DEVELOPMENT OF THE PROJECTION FROM THE EYE TO THE SUPERIOR COLLICULUS IN THE TAMMAR WALLABY (*Macropus eugenii*)

BY

DING, YUCHUAN (M.Sc., B. Med.)

A Thesis Submitted For The Degree Of Doctor Of Philosopy

Of

The Australian National University

Canberra, Australia

July, 1994

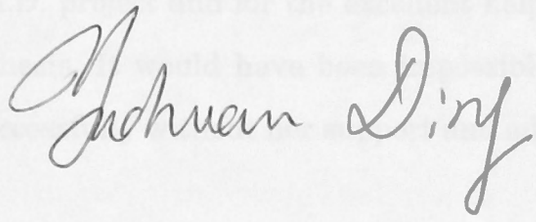
謹此獻給我的母親龐文淑女士

This thesis is dedicated to my mother, Pang Wenshu.

TABLE OF CONTENTS

Declaration

I declare here that all material in this thesis has not been submitted previously for a degree in any university and I believe that any material previously published or written by other persons is indicated by due reference in the text. This work was supported by an Australian National University Ph.D Scholarship and a Tuition Fee Scholarship.



Acknowledgements

First of all, I would like to give my most sincere thanks to Professor R.F. Mark, for being the best supervisor one could ask for, who allowed me access to the specific project working on the tammar wallaby and always gave me very helpful and friendly supervision.

I also wish to extend my many sincere thanks particularly to my supervisor, Dr L.R. Marotte for teaching me all the techniques which I used in the Ph.D. project and for the excellent help in all other aspects to produce this thesis. It would have been impossible for me to submit my Ph.D thesis successfully without her support and advice.

I am very grateful to Dr I.A. Hendry for his thoughtful suggestions on the preparation for the thesis.

Many thanks of mine are also given to Ms A. Devlin, Mr K. Williams for care and supply of pouch young wallabies, Ms M. Porter for her histological assistance and thoughtful reading of the thesis, and Mrs M. Donohue for her assistance in word processing.

My warmest appreciation goes to my wife Yan Ning for her company in my life, and encouragement and understanding during my Ph.D study.

Abstract

This thesis describes four studies on the development of the retinocollicular projection in the marsupial mammal, the tammar wallaby (*Macropus eugenii*).

(1). The time course of initial development of the retinal projection to the superior colliculus (SC). The anterograde tracer horseradish peroxidase (HRP) was injected into the eye *in vivo* in animals of various ages starting on the day of birth, to label retinal axons.

(2). The sequence of axon outgrowth from the developing retina to the SC. The fluorescent lipophilic carbocyanine dye, 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), was placed into the rostral SC *in vitro* in animals of various ages starting from 4 days after birth to retrogradely label ganglion cells projecting to the SC. The positions of labelled ganglion cells in wholemounts of the retina were recorded by a NeuroTrace computer system.

(3). The development of topography in the retinal projection to the SC. A small piece of Gelfoam impregnated with DiI was inserted into different quadrants of the retina to anterogradely label small groups of ganglion cell axons *in vivo* in animals from 8 days of age, onwards. Observations were made on wholemounts of the SC. The position of labelled retinal ganglion cell axons and their terminal zones was mapped by the NeuroTrace system.

(4). Retinotopic organization in the optic nerve during development. The optic nerves of animals used in (3) were examined and the position of labelled axons mapped.

The timing and sequence of events leading to the formation of the retinocollicular projection across the entire surface of the SC was established. At 4 days after birth, the first axons from the retina reach the contralateral SC at the rostrolateral border, and subsequently they spread caudally and medially over the SC. By 18 days, the axons reach the caudal pole and reach the medial border along most of the rostro-caudal extent except for the far caudal pole by 26 days. On the ipsilateral side, the first axons reach the rostrolateral edge of the SC at 5 days. They then spread over the SC also in a caudomedial direction and cover the rostrolateral part of the SC prior to 46 days. At 46 days after birth, the retinal projection covers the whole SC both contralaterally and ipsilaterally.

The outgrowth of ganglion cells from each quadrant of the retina which correlates with this sequence in the retinal innervation to the SC during development was demonstrated. At 4 days after birth, ganglion cells in dorsotemporal retina reach the contralateral SC first. This is followed by those from nasal and ventral retina at 9 days, with the region of retina projecting to the SC extending centro-peripherally. At 16 days, the first hint of a visual streak and area centralis in the retina is formed contralaterally. At 28-32 days, numbers of ganglion cells projecting to the SC reach a peak, with a qualitatively similar distribution to that seen at younger stages. From 41-46 days, a dramatic fall in the numbers of labelled ganglion cells is found in the retina although the distribution pattern of ganglion cells innervating the contralateral SC remains

unchanged. The ipsilaterally projecting ganglion cells are located initially in the central dorsal retina at 4-5 days and are then distributed diffusely over the retina up to 15 days. At 16 days, the beginning of the adult pattern is first seen, in which the ganglion cells become primarily restricted to the periphery of the temporoventral retina. The results suggests that the retinal projection, at least to the contralateral SC, may be topographically organized as it grows into the SC.

The degree of retinotopic organization in the contralateral and ipsilateral SC was investigated in more detail. From 8 to 40 days, developing retinal axons are distributed in a coarse topographic order. Beginning at 41 days, axons from the temporal retina begin to form their terminal arborizations in the correct topographic area in the rostral SC. From 52-55 day, axons from all retinal quadrants form their terminals in the topographically appropriate region although the more widely distributed labelled axons are still prominent. From 61-68 days, clearly defined terminal zones appear with the loss of more widely distributed axons. At 90-95 days, discrete terminals are present.

A similar coarse retinotopic order in the optic nerve was demonstrated at all stages of development. Temporal axons remain on the corresponding side of the optic nerve laterally and the nasal axons on the medial side. An inversion of axons from dorsal and ventral retina occurs. The axons gradually migrate from the corresponding dorsal and ventral regions towards the opposite side of the optic nerve. The order in the optic nerve may be sufficient to generate initially the coarse retinotopy of collicular innervation from dorsal and ventral retina, since the dorsal and ventral axons reverse their initial position in the optic nerve to enter the SC in the lateral and medial optic tract, respectively.

However, it can not explain the final retinotopy of synaptic connections between the optic nerve fibres and collicular neurons.

It has been concluded from the present study that there are two clear stages in the development of retinocollicular projections. A protracted period when retinal axons grow into the SC in coarse retinotopic order is followed much later by a period when the terminal arborizations are formed in the retinotopically appropriate position with the loss of more widely distributed axons.

Abbreviations

DiI	1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)
dLGN	dorsal lateral geniculate nucleus
HRP	horseradish peroxidase
LGN	lateral geniculate nucleus
NMDA	N-methyl-D-aspartate
SAI	stratum album intermedium
SAP	stratum album profundum
SC	superior colliculus
SGI	stratum griseum intermedium
SGP	stratum griseum profundum
SGS	stratum griseum superficiale
SO	stratum opticum
SZ	stratum zonale
TMB	tetramethylbenzidine
TTX	tetrodotoxin

Chapter 1. Introduction

GENERAL OVERVIEW

Wonder at the complexity of nature reaches its maximum when we consider the working of the brain. With this unlikely looking organ an animal plans its movements, decides on strategies of behaviour, regulates its internal to external environment, so as to adapt itself to the world around it.

Like other organs, the brain consists of relatively few basic cell types, neurons and three distinct class of supporting neuroglial cells. A fundamental characteristic of brain architecture, however, is that each of the more than 10^{10} neurons making up a mammalian brain has a distinctive function (Lund, '78). This function is determined not only by what class of neuron it is but also by its specific spatial distribution. The spatial distribution of specific neurons enables the individual neuron to receive a mass of information from a restricted and unique set of sources, and then transmit to another select group of cells. Specific connections are formed from the spatial interrelation of two interconnected regions and the topographic map of one region is delivered to the next.

The cellular organization and pattern of synaptic connections in the adult brain is the end result of an enormous number of changes having occurred during development. The processes responsible for the elaboration of the ordered synaptic connections that characterize the mature brain represent a striking feature of developmental neurobiology. The general problems and unravelling of mechanisms in the formation of neuronal connections by

which the organization and function of the adult brain is achieved has come to occupy a central position in neurobiological studies.

In attempts to elucidate the mechanisms controlling the formation of orderly nerve patterns, the visual system has proved to be most fruitful. Lund ('78) has summarized a number of reasons to demonstrate that the visual system is especially suitable for developmental studies. (1) the various parts are well circumscribed; (2) a map of the visual world is maintained in the visual areas of the brain, and this can be modified experimentally; (3) there is a considerable degree of dynamic interaction between its various components; (4) the eye is uniquely accessible to experimental manipulation that does not directly involve the rest of the central nervous system; and (5) the visual image can be more readily controlled than almost any other sensory stimulus.

Most understanding of the formation of visual connections in the mammalian brain is derived from studies on placental mammals, in which early development occurs in the uterus of the mother. The closed uterus and the intimate nature of the placenta, interposed for most of development between the embryo and its source of nourishment, makes it difficult, if not impossible, to investigate experimentally the early development of the brain. However, this does not apply to marsupial mammals. Marsupials, such as tammar wallaby, are born with an exceedingly immature central nervous system and there follows a protracted period of development in the pouch. The combination of birth relatively early along with an extremely long development in the pouch which is easy to access makes marsupials ideal for developmental studies on the neuronal connections. My thesis research focused on development of

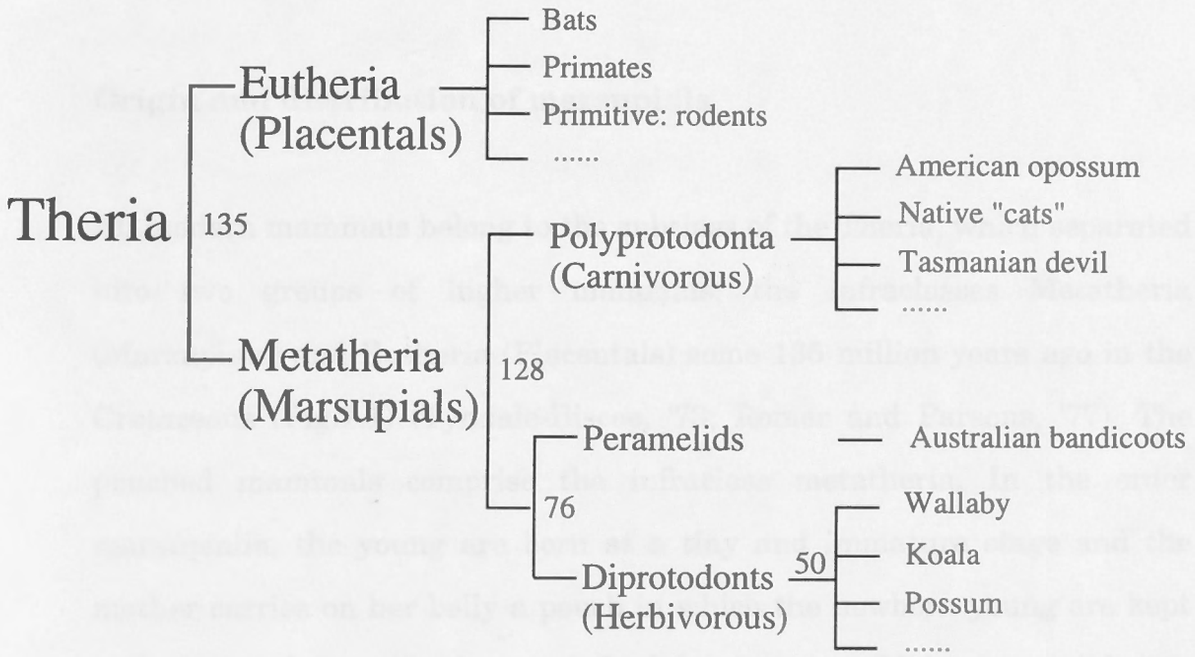


Figure 1.1 Diagrammatic family tree showing the evolution and relationships of the modern mammals. Numbers on the diagram indicate millions of years.

the projection from the eye to the superior colliculus and was carried out in wallaby *(Macropus eugenii)*.

LIFE OF MARSUPIAL WALLABY

Some important information on the life of the wallaby used in the present study as an experimental animal is set out below.

Origin and distribution of marsupials

All modern mammals belong to the subclass of the Theria, which separated into two groups of higher mammals, the infraclasses Metatheria (Marsupials) and Eutheria (Placentals) some 135 million years ago in the Cretaceous (Fig.1.1) (Tyndale-Biscoe, '73; Romer and Parsons, '77). The pouched mammals comprise the infraclass metatheria. In the order marsupialia, the young are born at a tiny and immature stage and the mother carries on her belly a pouch in which the newborn young are kept and nurtured for a further period of development. Male marsupials are normally pouchless and play little or no part in rearing the young.

Marsupials are currently found in two widely separated regions of the world, America and Australia. South America has been proved a haven for many marsupials during the Tertiary period some 65 million years ago, when that continent was isolated. A variety of marsupials (mainly carnivorous, such as Polyprotodonta) flourished there, almost all of them became extinct when the isthmian link to North America was re-establised and a host of more advanced mammals invaded that area. The American marsupials alive today are mostly small, ranging in size from mouse to rabbit, generally either carnivorous or omnivorous. Many live in forests

and feed mainly on insects (Stonehouse and Gilmore, '77). The American marsupials belong exclusively to the polyprotodont radiation.

Marsupials in Australia have elaborated considerably. By the Cretaceous this continent was separated from the rest of the world's land-masses and remained isolated. Few placental mammals had reached Australia at the time of separation and none have been able to reach it after, such that the marsupials had little opposition there. They expanded and diversified to fill almost every type of adaptive niche. The Australian metatherian (marsupial) mammals are classified in three orders, polyprotodonts, diprotodonts and peramelida. Polyprotodont marsupials include such pouched carnivorous forms as the native "cats" and the Tasmanian devil, the "native wolf", as well as many smaller insectivorous species such as the Dasywida. Diprotodonta with chisel-like front teeth are mainly herbivorous. Peramelida, intermediate between polyprotodonts and diprotodonts, include the Australian bandicoots (Romer and Parsons, '77). Macropodidae, the most advanced super family are derived from the suborder Diprotodonta. There are 56 living species at least in this group alone, and they are exemplified by the tammar wallaby (*Macropus eugenii*) (Kirsch and Calaby, '77).

Marsupials as typical mammals

Although always independent from placentals, the marsupials clearly possess the diagnostic features of typical mammals including high and stable body temperature, furry pelt, simple lower jaw and mammary glands. Especially, when considering the nervous system, comparative neurological studies have shown that they have neurologically representative mammalian brains in most respects. Most of the details of

neuroanatomy are so similar to those found in characteristic placental mammals that they can not be distinguished on histological grounds. There are differences within the marsupials, particularly between the more primitive ones such as American opossum and the advanced Australian diprotodonts such as tammar wallaby, but the differences between members of the placental and marsupial groups are in general no bigger than variations within either one. Parallels in the organization of the pyramidal tract, the auditory cortex, the visual thalamus, the somatosensory thalamus and visual cortex have been proved by electrophysiological experiments (Mark and Marotte, '92).

Reproductive method of marsupials

Marsupials, in most respects typical mammals, differ importantly from the placentals only in the method of reproduction, the most obvious being a short intra-uterine gestation followed by a protracted development in the pouch, outside of the mother's body. During the time in the pouch, the young are attached to teats of the abdominal mammary gland, nurtured by the mother's milk instead of a placental circulation. Neonatal marsupials, such as tammar wallaby, are born at less than 0.01% of the mother's weight and for the most part at a very early stage of development. Their physiological systems including the visual pathway develop during ensuing lengthy lactation (Tyndale-Biscoe and Janssens, '88). This slower maturation, nevertheless, follows the same sequence of developing organs, compared with most common placental laboratory mammals such as the rodent.

The fact that reproduction of the tammar wallaby is readily controllable makes for another experimental advantage. Wallabies are seasonal

breeding animals, with the first birth usually occurring in February and continuing to July. Embryonic diapause, in which development is suspended and can not be resumed even in the presence of apparently favourable conditions, is associated with a corpus luteum that is tonically suppressed by the pituitary, mediated by prolactin (Hearn, '73, '74). Two reproductive states have been termed lactational and seasonal quiescence (Tyndale-Biscoe et al., '74). During lactational quiescence from January or February to June in the Southern Hemisphere the corpus luteum is suppressed by sucking of the pouch young; removal of the young or denervation of the sucked gland will lead to reactivation. Females carrying a diapausing blastocyst will give birth and come into oestrus 26 to 28 days later (Renfree and Tyndale-Biscoe, '78; Renfree, '79; Merchant, '79). In seasonal quiescence from July to December, removal of the pouch young will not lead to reactivation of the dormant blastocyst. The dopamine agonist bromocriptine is known to depress the circulating level of prolactin in all species of mammals. Injection of 5 mg/kg wt of bromocriptine (Sandoz, Australia) early in seasonal quiescence will reverse this inhibition to the development of the corpus luteum. The embryo reactivates and birth of pouch young occur 26 to 28 days later (Tyndale-Biscoe and Hinds, '84). This feature of reproduction whereby the dormant blastocyst may be reactivated is particularly favourable to the early developmental studies since the harvesting of pouch young can be controlled on schedule.

Growth and development of the tammar

After 26-28 gestation days, the young tammar is born with a weight of 0.35 to 0.4 g. The neonatal animals remain permanently attached to one teat until day 90 to 100, during which time it grows slowly to reach a body weight of about 100 g and maturation of structure and function (Renfree et

al., '82). At about 140 days, the eyes open. By 160 days, the young is able to stand unaided and the pelage is thickening. At 180 days the young puts its head out of the pouch and nibbles grass. It is able to make its first excursion from the pouch at about 190 days. The young still needs to return to the mother to suckle for several more weeks until 250 days when it leaves the pouch permanently. It is then known as a runner.

PRIMARY VISUAL PATHWAY IN MARSUPIAL MAMMALS

The primary visual pathway in mammalian vertebrates is a part of the central nervous system. Organization of the visual system in marsupial mammals conforms to a plan which is essentially similar to that seen in placental mammals (for review see Mann, '64; Dowling, '70; Rodieck, '73; Johnson, '77; Lund, '78; Dunlop et al., '88; Mark and Marotte, '92). The visual system includes the retina, optic nerve, optic chiasm, optic tract, superior colliculus, lateral geniculate nucleus and visual cortex. The visual image is received in the retina, and transmitted through the optic nerve which comprises axons from retinal ganglion cells that converge at the optic disc to form the nerve. The optic nerve runs to the chiasm on the ventral surface of the forebrain. In the chiasm, some of the optic fibres from the retina of each eye cross the midline decussation and intermingle with axons from the other eye, while others remain on the same side. The degree of the decussation of fibres at the chiasm is probably related to the extent of binocular overlap depending on the placement of the eyes frontally or laterally in different species. Fibres crossed and uncrossed at the chiasm form the optic tract terminating in the primary visual centres. The retinal axons pass back around the side of the brainstem to enter the dorsal lateral geniculate nucleus (dLGN), the superior colliculus (SC, the homologue of the optic tectum in non-mammalian vertebrates) and the

various pre-tectal and accessory optic nuclei. The dLGN displays a feature of mammals, in which there is a pronounced lamination of cells and fibres with its separated eye-specific bands. Cells in the dLGN send axons to the visual cortex, which end in different layers of the cortex, primarily in layer IV.

Tracing the axons of retinal ganglion cells from their receptive fields in the retina to the primary target, the SC has provided a great deal of information about how specific connections concerned with topographic specificity are formed, and how developmental synaptogenesis extends to precise connections between ganglion cell terminals and the cell bodies of the SC. Anatomical features of the primary visual pathway including retina, ganglion cells, optic nerve and the SC, involved in the present study, will now be described in detail.

Cellular structure of the retina

Anatomical analysis on the retina of marsupials (for review see Johnson, '77; Dunlop et al., '88) shows that its cellular structure follows the same general plan for all mammalian vertebrates, which was first demonstrated in the pioneering work of Roman y Cajal (1892, 1911). The mature retina consists of a thin plate having three cellular layers (the outer and inner nuclear layers and ganglion cell layer), that are separated by two plexiform layers (the outer and inner plexiform layers). The cellular layers contain morphologically and functionally distinct neurons, in which the outer nuclear layer contains the photoreceptors including rods and cones; and the inner nuclear layer contains all horizontal bipolar and interplexiform cells, most amacrine cells and a few ganglion cells; the ganglion cell layer contains the cells bodies of most ganglion cells as well as a large number of

displaced amacrine cells. Synaptic connections between these neurons are made in the outer and inner plexiform layers. A visual image is received in the retina by photoreceptors on the outer nuclear layer at the very back of the eye, passing through the optics of the eye, such as cornea, aqueous humor, pupil, lens and vitreous body. The image is relayed through the bipolar cells by means of synaptic connections with photoreceptors in the outer plexiform layer, to the ganglion cells in the ganglion cell layer. Two sets of interneurons, horizontal cells and amacrine cells modify the input at the relay points (Dowling, '70). The ganglion cells are the only retinal neurons to send axons to the central retinorecipient nuclei, such as the SC.

An avascular retina, which relies on a well developed choroidal circulation to supply nutrients and oxygen diffusion is a feature in marsupials although not restricted to them (Johnson, '01; Freeman and Tancred, '78; Chase, '82; Dunlop et al., '88) A few fine capillaries arise from the optic disc and radiate a short distance across the nerve fibre layer in kangaroos and phalangers (Johnson, '01), and a more extensive retinal circulation is found in the retina of native cat (Dunlop et al., '88). Variations of the proportions of choroidal and of retinal circulation are also documented in placental mammals. The retinal vessels cover the entire retina in human, monkey, cat and rat, while an avascular retina is found in guinea pig (for review see Stone and Dreher, '87; Dunlop et al., '88). Rabbit provides an example of partial vascularization with vessels being confined to the myelinated visual streak and the nutrition of the rest of the retina being provided by the choroidal circulation (Davis, '29; Chase, '82; Stone and Dreher, '87).

Ganglion cell layer

There are two main neuronal types, ganglion cells and displaced amacrine cells in the ganglion cell layer in marsupial mammals as well as in placental mammals (Rodieck, '73; Hughes, '85; Dunlop et al., '88). Ganglion cell axons run across the ganglion cell layer in the innermost surface of the retina and converge at the optic disc, where they leave the eye and enter the optic nerve. Displaced amacrine cells comprising the second cell type are intrinsic retinal neurons with no axon leaving the eye.

The distribution of ganglion cells across the eye has been studied in both marsupial and placental mammals. The retina of most adult marsupials, like many placental mammals, has a well-developed visual streak containing a temporally placed area centralis, which has the highest density of ganglion cells. This topographic organization of ganglion cells is specialized to various degrees. Such specializations may reflect an animal's habitat (Hughes, '77). In species such as tammar wallaby (Wong et al., '86) and grey kangaroo (Dunlop, et al., '87), ganglion cells are distributed in a pronounced, horizontally aligned visual streak with a high cell density. This visual streak is positioned superior to the optic disc, although one exception is demonstrated in koala that has a visual streak inferior to the optic disc (Schmid et al., '92). Peak cell counts are found within the temporal arm of the visual streak in a small, circular, oval or cruciform region termed the area centralis. Ganglion cell densities decline markedly above and below the visual streak. An area centralis, associated with a relatively poor visual streak, has been reported in tree kangaroo (Hughes, '74), brush tailed possum (Freeman and Tancred, '78), brown bandicoot (Tancred, '81), northern native cat (Harman et al., '86), koala (Schmid et al., '92) and quokka wallaby (Beazley and Dunlop, '83).

The distribution of retinal ganglion cells in placental mammals also shows a wide range of topographies. Ganglion cells of most species are distributed across the retinal surface in a non-uniform pattern. There are areas of elevated cell density such as the visual streak in rabbit (Oyster et al., '81; Stone, '83) and the area centralis in cats (Stone, '83; Hughes, '85) and rodents (Sengelaub et al., '86; McCall et al., '87). In primates, topographic distribution of retinal ganglion cells is characterized by a fovea (Stone and Johnston, '81; Provis et al., '83). A significant centro-peripheral gradient in ganglion cell density is found in the rodent (Tiao and Blakemore, '76; McCall et al., '87). In the cat and primates such as monkey and human, the centro-peripheral ratio of density is even greater (Van Buren, '63; Stone, '65; Rolls and Cowey, '70; Hughes, '75; Stone and Johnston, '81; Perry and Cowey, '85; Lia et al., '87).

The number of ganglion cell in the ganglion cell layer has been counted in both marsupial and placental mammals. In the grey kangaroo, quokka wallaby, koala and tammar wallaby, ganglion cells comprise 40%, 56%, 60% and 65% of neuronal population in the ganglion cell layer, respectively. There are about 280,000 ganglion cells in the retina of the brush-tailed possum (Freeman and Tancred, '78). Values of 101,026 for total ganglion cells in each eye are recorded in North American opossum, values of 532,800 in grey kangaroo, values of 360,000 in tammar wallaby and values of 201,000 in quokka wallaby (Rapaport et al., '81; Beazley and Dunlop, '83; Wong et al., '86; Dunlop et al., '87). For placental mammals, the proportion of ganglion cells varies more widely between species, being 19% in cat (Wong and Hughes, '87), 50-60% in rat (Perry et al., '83) and 63% in rabbit (Vaney et al., '81; Hughes, '85). The number of ganglion cells was also obtained, being 100,000-115,000 in rat (Perry, '81, Perry et al., '83;

Linden and Perry, '82; Potts et al., '82; McCall et al., '87), 151,000-170,000 in cat (Chalupa et al., '84; Wong and Hughes, '87; Robinson, '91), 90,000 in ferret (Henderson et al., '88), 291,000 in rabbit (Robinson et al., '87), and 1.41-1.81 million in monkey (Fischer and Kirby, '91).

Studies on development of ganglion cells mainly concern features of the topography of ganglion cells such as the formation of the fovea or the area centralis and visual streak. Pioneering studies on development of the ganglion cell layer was carried out about 100 years ago in human retina (for review see Mann, '64; Dunlop et al., '88). A fovea and a centroperipheral gradient in ganglion cell density was found to form gradually from a uniform distribution of ganglion cells (Mann, '64; Provis et al., '83). Such studies in quokka wallaby and grey kangaroo (Beazley and Dunlop, '83; Dunlop and Beazley, '85; Dunlop et al., '87) show that initially the distribution of total cells or ganglion cells in the ganglion cell layer are approximately uniform and that an adult-like topography with the appearance of the visual streak and area centralis is present later on. This transition from an approximately uniform distribution of cells to the adult topography seems to be a prominent feature of mammalian retinal development since it is also demonstrated in other mammals such as cat (Stone et al., '82; Kelling et al., '89), rat (McCall et al., '87) and ferret (Henderson et al., '88) as well as human (Mann, '64; Provis et al., '83). The mechanisms which establish these typical retinal specializations appear to make use of a variety of factors including the differential generation of cells, the selective death of cells in the ganglion cell layer, and the disproportionate areal growth of retina (Coulombre, '56; Stone et al., '82; Rapaport and Stone, '83; Mastronarde et al., '84; Lia et al., '87; Sengelaub et al., '86; Dunlop et al., '87; Dunlop and Beazley, '87; Harman and Beazley, '87; Dunlop et al., '88; Henderson et al., '88; Kelling et al., '89).

Optic nerve

The ganglion cell axons run along the inner surface of the retina and gather together to form the optic nerve, as they exit the eye. The optic nerve is the second cranial nerve and like the retina is a part of the central nervous system by embryonic origin.

In the adult optic nerve, axon numbers are found to be in close agreement with retinal ganglion cell counts in marsupials such as brush tailed possum (Freeman and Watson, '78), North American oposssum (Kirby et al., '82), quokka wallaby (Braekevelt et al., '86), as well as in eutherians such as rat (Lam et al., '82; Perry et al., '83; Sefton and Lam, '84; Crespo et al., '85), cat (Williams et al., '83, '86; Robinson, '91) and rabbit (Robinson et al., '87). It appears that only ganglion cells in the retina send axons to the central target and each retinal ganglion cell has a single axon in the optic nerve.

Over-production and subsequent loss of axons during development have been reported in similar patterns amongst mammalian vertebrates, including a number of marsupials such as quokka (Braekevelt et al., '86), North American opossum (Kirby et al., '88) and Australian native cat (Crewther et al., '88) as well as other mammals such as rat (Lam et al., '82; Perry et al., '83; Sefton and Lam, '84; Crespo et al., '85), hamster (Tay et al., '86), rabbit (Robinson et al., '87), cat (Ng and Stone, '82; Williams et al., '86), monkey (Rakic and Riley, '83) and human (Provis et al., '85). The time course of these changes in the numbers of axons in the optic nerves is variable in different mammalian species. These studies have been made in marsupial mammals such as quokka wallaby, and in placental mammals such as human, monkey, rabbit, cat, hamster, and rat (for review see

Dreher and Robinson, '88). The time-course of changes in the numbers of optic nerve axons varies dramatically from one species to another, occuring either in the middle of the gestation period or towards the end of the gestation period, and in some cases the changes even occur postnatally. However, when compared as a proportion of the "caecal period" or blind period ("CP", the interval between conception and natural eye opening), the changes in the number of axons are found to follow a roughly similar relative time course in those mammalian species studied. Although, during the first half of the caecal period, most events occur earlier in marsupials than in eutherians, most events occur at the same stages of the caecal period in both marsupials and eutherians during the second half of the caecal period. The accelerated rate of visual development during the first half of the caecal period in marsupials may be related to the precocious development that they undergo prior to their very early birth (Robinson and Dreher, '90). Possible factors underlying the reduction of axon numbers in the optic nerve are thought widely to be the death of ganglion cells rather than the retraction of axonal collaterals (Cunningham et al., '82; Sengelaub and Finlay, '82; Stone et al., '82; Potts et al., '82; Dreher et al., '83; Lia et al., '87; Perry et al., '83; Sengelaub et al., '86; McCall et al., '87; Robinson et al., '87, '89).

As in placental mammals, in adult marsupials studied so far, almost 100% of fibres in the optic nerve are found to be myelinated in brush tailed possum (Freeman and Watson, '78), North American oposssum (*Didelphis virginina*, Kirby et al., '82), quokka (Beazley and Dunlop, '83; Braekevelt et al., '86) and grey kangaroo (Dunlop et al., '87), while 80% of fibres are found to be unmyelinated in opossum (*Didelphis marsupialis,* Hokoc and Oswaldo-Cruz, '78). In comparison with eutherians, the development of myelination in marsupial optic nerve is different in that it is very

protracted. In the quokka, for example, the myelinated axons are first seen at 85 days after birth, with only 15% of axons being myelinated by 130 days and 76% by 150 days. In contrast to quokka, however, 3% of axons in cat optic nerve are myelinated at birth, 22% by 8 days and 93% by 30 days (Ng and Stone, '82). In rat optic nerve, 85% of axons are myelinated within 3 weeks after birth (Foster et al., '82). The onset of myelination also appears to be less rapid in marsupials than in eutherians. Partially wrapped axons are found initially in quokka wallaby (Braekevelt et al., '86), while few are seen in cat during early development (Moore et al., '76). Electron microscopy reveals, in the quokka, the presence of compacted strips of myelin-like membranes about 40 days before the first myelinated axons are seen (Dunlop et al., '88). In postnatal rat, however, compacted membranes partially enclose bundles of unmyelinated axons less than one week before the first differentiated nodes of Ranvier are observed (Hildebrand and Waxman, '84). Conflicting sequences of myelination along the length of the developing optic nerve have been demonstrated in marsupials and eutherians on morphological studies. A preliminary study suggests that myelination of the optic nerve axons is initiated from the chiasm rather than the eye (Dunlop et al., '88). In contrast, contradictory results were obtained from studies on various eutherians (for review see Moore et al., '76). A gradient of myelination from the brain towards the eye is revealed in optic nerve of human and kitten (Bembridge, '56; Banik et al., '68; Blunt et al., '72). However, myelination was found to be formed first near the retina and then proceed centrally in a study on kitten (Moore et al., '76).

Anatomy of the superior colliculus

The primary visual brain centre in non-mammalian vertebrates is the optic tectum, which receives visual input and assists generating visually guided behaviour. The evolution of the cerebral cortex in mammalian vertebrates did not result in the elimination of the tectum, instead as the superior colliculus (SC), this visual centre continues to play a function in the organization of visually directed behaviour (Sprague et al., '73; Ingle, '73).

The mammalian SC is positioned caudal to the thalamus in the midbrain, bordered rostrally by the pretectum, caudally by the inferior colliculus, ventrally by the mesencephalic central gray and reticular formation. The SC is a layered structure, which is divided into seven alternating fibrous and cellular layers. The layers comprise from dorsal to ventral as follows: stratum zonale (SZ), stratum griseum superficiale (SGS), stratum opticum (SO), stratum griseum intermedium (SGI), stratum album intermedium (SAI), stratum griseum profundum (SGP), and stratum album profundum (SAP). The layered structure is usefully viewed as being composed of two main subdivisions: superficial and deep (Harting et al., '73). As is commonly done the three dorsal layers will be referred to as the superficial layers and the four ventral layers will be referred to as the deep layers. The layered system is found in all mammals studied (Ramon y Cajal, '11; Olszewski and Baxter, '54; Viktorov, '66; Lund, '72; Stein, '81), including marsupials (Pearson et al., '76; Royce et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79; Wye-Dvorak, '84; Sheng et al., '90).

The superficial compartment of the SC is dominated by inputs from the retina and visual cortex, which appear to be concerned with the processing of visual information. Afferents primarily from the retina have been found

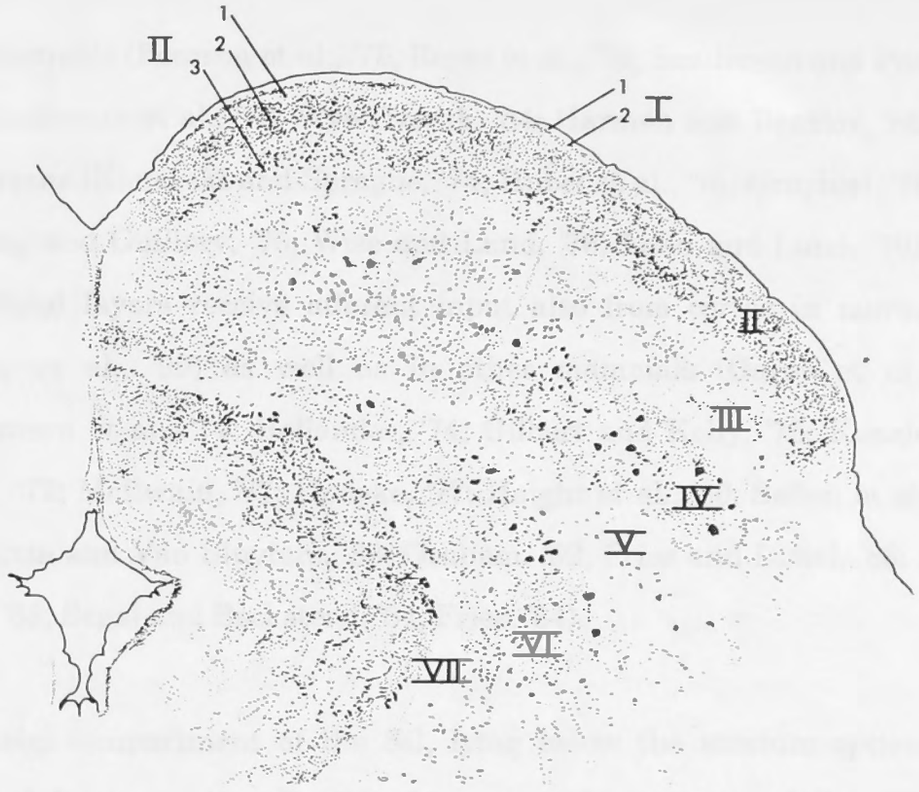


Figure 1.2A Projection drawing of Nissl-stained section of cat superior colliculus illustrating laminar organization. Dots correspond to collicular neurons and approximate size. Numbers indicate the different laminae and sublaminae (Reproduced with permission from Kanaseki and Sprague, '74; Goldberg and Robison, '78). I: SZ; II: SGS; III: SO; IV: SGI; V: SAI; VI: SGP; VII: SAP.

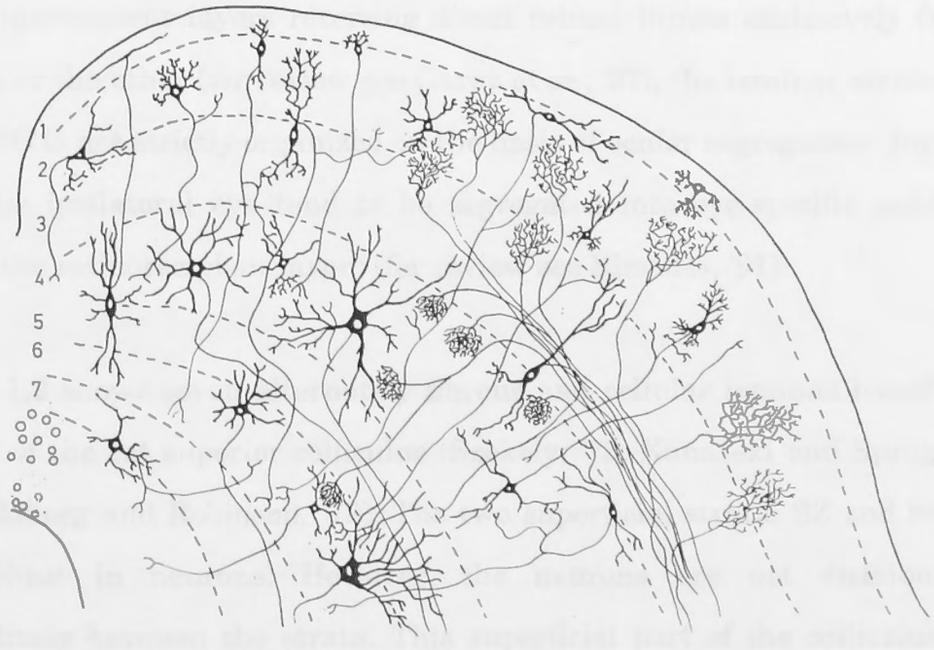


Figure 1.2B Diagrammatic representation of the cat's superior colliculus (Szekely, '73). Numbers indicate the different strata (same as above).

in marsupials (Pearson et al., '76; Royce et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79; Wye-Dvorak, '84; Harman and Beazley, '86) and eutherians (Kanaseki and Sprague, '74; Hubel et al., '75; Graybiel, '75, '76; Harting and Guillery, '76; Wise and Lund, '76; Land and Lund, '79). The superficial layers receive afferent input also from cortex in marsupials (Sheng et al., '90) as well as in other mammals (Garey et al., '68; Kawamura et al., '74; Hollander, '74; Gilbert and Kelly, '75; Kunzle and Akert, '77; McIlwain, '77; Updyke, '77; Haight et al., '80; Sefton et al., '81; Olavarria and Van Sluyters, '82, Graham, '82; Fries and Distel, '83; Segal et al., '83; Segal and Beckstead, '84; Fries, '84).

The deep compartment of the SC, lying below the stratum opticum, is occupied by non-optic afferents from several sensory modalities, motor areas, and areas that are neither purely sensory nor purely motor. These come both from cortical and subcortical sources (for review see Sparks, '86). Unlike the LGN, in which there is an overt cellular lamination with each of the retinorecipient layers receiving direct retinal inputs exclusively from one eye or the other (for review see Garey et al., '91), the laminar structure in the SC is not strictly organized on the basis of ocular segregation. Inputs from the ipsilateral eye tend to be segregated into eye-specific patches within the retinorecipient layers (for review see Rhoades, '91).

Figure 1.2 shows seven alternately fibrous and cellular laminae based on studies of the cat superior colliculus (Szekely, '73; Kanaseki and Sprague, '74; Goldberg and Robinson, '78). The two superficial strata, SZ and SGS, are richest in neurons. However, the neurons are not distributed distinctively between the strata. This superficial part of the colliculus is the principal recipient of optic afferents. In addition to optic axons, some corticocollicular projections terminate in this part and a number of

ascending axons of deeper lying neurons target here. The SO is a lamina of white matter with indistinct borders containing a few smaller neurons. The superficial part of this layer contains the optic axons which arrive from the optic tract. These axons terminate towards the surface in the overlying stratum. The SGI is relatively wide, dominated by large multipolar neurons. In addition, a number of small and medium size neurons of diverse form make the cell population in this stratum variegated as in the superficial strata. The axons terminate in the neighbouring strata, some of them ascending as far as the SZ. The SAI contains the myelinated fibres of large multipolar neurons, and is the main efferent pathway of the tectum. A few small and medium size neurons make the borders of this layer indistinct from the neighbouring strata. Although the SGP is not well delimited, the neuron population is similar to that of the SGI. The SAP delimits the colliculus from the central gray matter. It contains the axons of overlying neurons and commissural fibres which interconnect the two colliculli.

Little information is available on the developmental laminar structure in the SC of marsupials. A study on brush tailed possum shows that at 9-10 days after birth the SC is not differentiated into layers and there is a thick zone of cell proliferation around the ventricle. By 23 days, the well-defined cell layers in the SC are formed, with some indication of cell proliferation around the ventricle until 40 days (Sanderson et al., '82). In the wallaby, the superficial layers of the SC including the SZ, the SGS and the SO can be first identified on the basis of cytoarchitectonics from 81 days after birth, although they are not as distinct as in the adult (Sheng et al., '90).

Topography of retinocollicular projections

The fact that certain nuclei receive direct topographically ordered projections from the retinal ganglion cells is a feature in the brain of vertebrates. One of their main destinations is the superior colliculus (or optic tectum in non-mammalian forms), over which the temporal retina projects rostrally, the nasal caudally, the dorsal laterally and the ventral medially (Kruger, '70; Schiller, '84; Flett et al., '88; Mark et al., '93a). Three strategies of mapping have been demonstrated in the retinotectal projections (for review see Lund, '78; Flett, '86; Flett et al., '88; Mark et al., '93a). First, the retinotectal projection has a map of the entire contralateral retina with all axons crossing at the optic chiasm. This organization is found in non-mammalian vertebrates which have lateral eyes such as fish, amphibians and birds (Campbell and Ebbesson, '69; Scott, '73; Clarke and Whitteridge, '76). Amongst the mammals, the decussation plan is complicated by a direct pathway from the ipsilateral eye and by differences in the amount of contralaterlal retina having a collicular projection. In rabbits, which have laterally placed eyes, there is almost complete decussation of the optic nerve so the projection to the SC is almost entirely of the contralateral hemifield (Hughes, '71). However, in the primates such as monkey, the eyes are in the frontal plane, the nucleus in the SC has a map formed through partial decussation of the optic nerve, in which it receives input from the contralateral retina nasal to the vertical midline and from the ipsilateral retina temporal to the midline. The contralateral visual hemifield is represented in the nucleus and most collicular visually responsive neurons are binocular (Cynader and Berman, '72; Lane et al., '73; Stone et al., 73). This is the second strategy of organization. In between, there are many variations combining a complete representation of the field of the contralateral eye with more or less of the ipsilateral retinal

contribution, generally from the temporal retina, to a binocular region. A visual field that extends from the far periphery contralaterally to beyond the vertical midline is represented in the colliculus (Lund et al., '74; Guillery, '82; Holcombe and Guillery, '84; Insausti et al., '84). Apart from megabats and primates, this third strategy of mapping retinal projections to the SC is employed by all placental mammals studied so far in which the eyes may more or less be lateral (for review see Flett, '86). Marsupial mammals such as American opossum and tammar wallaby follow this plan (Volchan et al., 82; Flett et al., 88). Although a variety of factors can influence the laterality of retinal projections to the SC, the relationship beween the extent of the partial decussation at the optic chiasm and the position of the eyes and the extent of a binocular frontal field has been demonstrated (Lund, '78; Guillery, '82).

The anatomical background of the primary visual pathway in marsupials, in comparison with that in eutherians has been reviewed and their similarities noted. The general plan of the marsupial visual system during development conforms to that of eutherians. Marsupials therefore provide an excellent opportunity for studies on the mechanisms underlying the formation of neuronal connections in the brain. The results of experiments with these animals are relevant to experimental work on traditional laboratory mammals and eventually to the structure, function and development of the SC in human beings.

Outline of the project and the methods used

Working out the processes of formation of topographic retinocollicular connections and the mechanisms, by which the topography of the retinal

projection in the superior colliculus (SC) of the wallaby is set up, is the major aim of my Ph.D thesis research.

First of all, a study on the time course of initial development in the retinal projections to the SC was carried out. The pattern of retinal innervation to the SC initially in development and the subsequent distribution of the whole population of axons on the SC is described **in chapter 2**. This involved using the anterograde tracing technique with the enzyme horseradish peroxide (HRP).

With the knowledge of the sequence of retinal innervation in the SC, which part of the retina projects to the SC first and the subsequent order of axonal outgrowth from different quadrants of the retina became the focus for **chapter 3**. This study involved using a retrograde tracing technique, with the fluorescent lipophilic carbocyanine dye 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) *in vitro*. DiI provides intense and long-lasting staining of neurons in embryonic and neonatal brain tissue. Neuronal processes can be labelled along considerable distances in both anterograde and retrograde directions. This dye is capable of diffusing in the lipid bilayer of aldehyde-fixed cell membranes (Godement et al., '87; Honig and Hume, '89). The labelling observed in fixed tissue is restricted to the plasma membrane of neurons and the labelling spreads further along axons with time. Instead, granular intracellular labelling occurs in living tissue in which staining is totally due to active axonal transport of the internalized membrane vesicles, and this extends much faster over distances than in fixed tissue.

In chapter 4, the establishment and possible mechanisms of topographic organization of individual retinal axons in the SC is reported by using DiI

as an anterograde tracer *in vivo*, in which the staining is intracellular and rapid over quite long distances.

The retinotopic order in the optic nerve and its relationship to the establishment of the visual map during development are described **in chapter 5** by using DiI as an anterograde tracer *in vivo*.

Finally, some general remarks and conclusions from these studies are made **in chapter 6.**

Chapter 2. Time Course Of Initial Development Of The Retinal Projection To The Superior Colliculus

INTRODUCTION

Observations on the time-course of development of retinal projections to the SC have been carried out extensively in non-mammalian vertebrates. A rostrocaudal sequence of ingrowth in retinal innervation to the tectum (the homologue of the SC in mammals) was obtained in frog (Gaze et al., '74; Currie and Cowan, '75; Holt, '84), fish (Stuermer,'88a) and chick (De Long and Coulombre, '65; Goldberg, '74; Crossland et al., '75; Rager and von Oeynhusen, '79; Thanos and Bonhoeffer, '83; Fujisawa et al., '84; McLoon, '85). In addition, studies on the pattern of projections from the eye to the SC have been made widely in adult and developing mammalian vertebrates such as in placental mammals: monkey (Rakic,'77), rat (Land and Lund, '79), hamster (Frost el al., '79; Jen et al., '84), mouse (Godement et al., '84; Edwards et al., '86), cat (Williams and Chalupa,'82), grey squirrels (Cusick and Kaas,'82), rabbit (Gayer et al., '89; Crabtree, '89), and in marsupials: opossum (Cavalcante and Rocha-Miranda,'78), brushtailed possum (Pearson et al., '76; Sanderson et al., '78, '82), quokka (Harman and Beazley,'86) and kowari and fat-tailed dunnart (Haight and Sanderson, '88). However, although these studies in mammals involved the time course of retinal innervation to primary visual centres, there was not much detailed information on time course and spatial pattern of the initial innervation to the superior colliculus (SC).

The time-course of innervation to the SC at closely spaced time intervals remains largely unknown in mammals. Understanding of the time course of ingrowth of the retinal projection to the SC in mammals mainly comes from the *in utero* studies on the rodent and from a study of pouch young in the tammar wallaby. In rat, the first axons enter the optic chiasm at embryonic day 15 and by 16 days the axons have reached the SC rostrally. At 17 days, axons have expanded across the entire rostrocaudal extent (Lund and Bunt, '76). Development of retinocollicular projections was also obtained in prenatal and postnatal mice (Godement et al., '84; Edwards et al., '86). Initial fibres enter at the rostral and lateral edges and extend rostrocaudally across the surface of the contralateral SC. After a short delay, uncrossed fibres spread in a similar pattern ipsilaterally. Innervation ipsilaterally is absent caudally later on, as in mature animals. In these species, however, the duration of the period from when the first retinal axons reach the SC, to when the retinal projection grow across the target, is very short (Lund and Bunt,'76; Godement et al., '84; Edward et al., '86). Thus, a more detailed time-course of the pattern of ingrowth of the retinocollicular projection was not available. A time-course and pattern of optic innervation on early development of the retinocollicular pathway was obtained in the tammar wallaby by using autoradiography after labelling optic axons with a tritiated amino acid (Wye-Dvorak, '84). Axons first invade the contralateral SC from 9 to 12 days after birth, and first invade the ipsilateral SC from 21 days as the label in the contralateral SC extended almost to the medial border. By 30-35 days, the retinal projection extends over the SC contralaterally, while the label is lighter and confined to the rostral pole of the SC ipsilaterally. At 52 days, the light ipsilateral label is restricted in the deeper layer. At 63-64 days, the ipsilateral label lying beneath the superficial layer becomes patchy. At 72 days, the pattern of label is similar to the adult, in which distinct cellular layers are

apparently formed in the contralateral SC and label is concentrated in the rostral pole of the ipsilateral SC.

The main aim of the present experiment reported here is to determine the detailed time course of ingrowth of primary visual projections to the SC in tammar wallaby early in development, using the more sensitive HRP (horseradish peroxidase) technique. This method of anterogradely labelling retinal axons from the eye does not have the problems of the autoradiographic method in trying to see sparsely labelled axons against background label early in development.

Developing optic axons were labelled from the earliest postnatal stage (day of birth) to the age when optic axons cover the entire superficial layers of the SC both contralaterally and ipsilaterally. When the first crossed and uncrossed retinal axons reach the SC and how they grow subsequently across the SC with age will be described.

MATERIALS AND METHODS

Animals

Eighteen animals *(Macropus eugenii)* aged between 0 (birthday is regarded as 0 day) and 46 days, at intervals of 1 to 5 days, were obtained from the RSBS wallaby colony at the ANU campus. Animals aged at 0, 2, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 23, 26, 29, 35, 39 and 46 days were of exactly known birthday.

HRP injections

Pouch young were anaesthetized by hypothermia in ice. A dorsal triangular flap of eyelid was cut, and a hole, localized at margin of the pupil, was made temporally in one eye with a sharp glass pipette. An intravitreal injection of 1-2 µl of a 30% solution of HRP (Sigma, Type VI) was made slowly into the eye of the pouch young. The eyelid flap was repositioned by suture. The pouch young was warmed up by keeping it in a humid incubator for 10 to 20 minutes. When the forearms and body were moving vigorously, the animals were reattached to the teat in the pouch. The mother was anaesthetized briefly with 2.5-4 ml of 2.5% brietal sodium (Lilly), injected into the lateral tail vein during this procedure.

Histological procedures

After 24 hours survival, the animals were deeply anaesthetized by cooling and briefly perfused intracardially through a microcapillary pipette with warm physiological saline (0.9%), followed by a cold solution of 1% paraformaldehyde/1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 minutes. 10% sucrose in 0.1 M phosphate buffer was then perfused for another 30 minutes. The brains were dissected out and stored in 20% sucrose in 0.1 M phosphate buffer at 4^0 C overnight. On the following day, brains were immersed in gelatin-albumin for 20 minutes, and then embedded in gelatin-albumin hardened with glutaraldehyde for 20 minutes. The ratio of gelatin-albumin and glutaraldehyde was 10:1. On a freezing microtome, brains of animals were sectioned at a thickness of 40 µm, in the frontal plane throughout the rostral-caudal extent. All sections were collected serially onto 0.1 M phosphate buffer (pH7.2-7.4) in perforated trays and rinsed in one change of this buffer. Subsequently, the

sections were processed according to the TMB method (Mesulam,'82). Two series of sections, A and B were then mounted separately on gelatinized slides. The sections were dried at room temperature. B series was counterstained with 0.05% thionin for 5 minutes. Both A and B sections were then dehydrated in graded alcohols, cleared in xylene and mounted in depex.

Analysis

Sections were observed in a Leitz Orthoplan microscope. At every second section of series A, an outline of the transversely sectioned SC and HRP-labelled axons were drawn using a camera lucida at a magnification of 60 times. Either bright-field or dark-field illumination was used for each section. The borders of nuclei were detected with the aid of series B stained by thionin and the distribution of labelled retinocollicular fibres was traced with the aid of the unstained series A. Subsequently, a three dimensional reconstruction of the SC revealing the distribution of the retinocollicular projections on the contralateral and ipsilateral side was made.

RESULTS

The age of the animal referred to in the results is the age at the time of injection. However, it should be kept in mind that the optic projection presumably continues to grow after the injection of the tracer, during the period of survival. Artifacts of crystals were seen on the surface of the brain because no attempt was made to remove the dura in these young animals due to the danger of damaging retinal axons as they crossed the

surface. Removal of the dura greatly reduces this artifact (L.R. Marotte, personal communication).

The retinal projections to the SC in adult (Wye-Dvorak, '84) showed that retinal axons enter the stratum opticum (SO) of the SC contralaterally. The superficial gray layer (SGS) above this receives a heavy projection, while the outermost layer, the stratum zonale (SZ), receives a light projection. The ipsilateral projections are confined to the rostral half of the SC, showing as a series of discrete clusters in the intermediate gray layer (SGI) beneath the SO. This mature pattern of retinal projection begins to be present from 72 days after birth.

All the figures for this chapter are grouped together at the end of the results section.

0 day (n=1)

Retinal fibre projection. Labelled axons were only found at the optic chiasm but none were found beyond the chiasm in the optic tract.

2 days (n=1)

Retinal fibre projection. Some contralateral axons crossed the chiasm and ran parallel to the ventrolateral diencephalic surface (Fig.2.1A, B). The fibre fascicles in the optic tract extended along the lateral surface of the diencephalon to reach the level of the pretectal area, but disappeared before reaching the rostral pole of the SC (Fig.2.1C-F). On the ipsilateral side, a very few axons were spread over the surface of the diencephalon laterally and ventrally.

4 days (n=1)

Retinal fibre projection. More labelled axons than those seen at 2 days could be seen at the optic chiasm and coursing toward visual areas in the contralateral thalamus and midbrain. Many fine HRP filled optic fibres were present along the contralateral optic tract and ran over the surface of the dorsal lateral geniculate body (Fig.2.2A, B). Labelled contralateral fibres were first visible in the rostral SC ventrolaterally by this stage although they were only distributed sparsely (Fig.2.2C, D).

Distribution of retinal projection in the SC. In three dimensional structure (Fig.2.3), the retinal axons were found to be distributed in the SC rostrolaterally. Ipsilaterally very few fibres appeared on the ventrolateral surface of the diencephalon but had not entered the SC.

5-6 days (n=2)

Retinal fibre projection. From this stage, large numbers of fibre fascicles from the optic tract began to invade the contralateral SC. Many labelled fibres extended across the surface of the SC and some finer HRP label was first seen laterally and rostrally beneath these labelled fibres. The axons passed rostrocaudally along the ventrolateral aspects of the collicular surface just underneath the pia. Label in the superficial layer decreased in density toward the caudal pole (Fig.2.4). Ipsilaterally, sparse fibres were seen within the optic tract and a few of them began to invade the rostral pole of the SC. Because the label is extremly sparse, no picture is available, showing this clearly.

Distribution of retinal projection in the SC. The distribution of labelled fibres occupied the lateral half of the contralateral SC and extended over most of the rostral-caudal extent, with the exception of the caudal pole. No fibres were close to the medial border. Ipsilaterally, retinal axons were distributed in a very small portion of the rostrolateral SC (Fig.2.5).

8 days (n=1)

Retinal fibre projection. Contralaterally, the coarse superficial label became thicker and the finer label beneath it increased in depth (Fig.2.6).

Distribution of retinal projection in the SC. The SC had increased in size both in rostral-caudal and lateral-medial extent. The extent of the contralateral retinocollicular projection was similar to that seen at earlier stages. Labelled axons covered the lateral contralateral SC throughout the rostrocaudal extent. Denser superficial label was seen rostrally, while sparser label was seen caudally. Ipsilaterally, sparse label was found at the rostral-lateral pole of the SC. The label had extended slightly more caudally and medially, compared to that seen at 5 days (Fig.2.7), but very few fibres were present compared to the contralateral side.

10-12 days (n=2)

Retinal fibre projection. Coarse retinal fibres were prominent superficially over almost the whole rostral-caudal extent of the contralateral SC, especially rostrally (Fig2.8A-F). Finer HRP reaction product deep to this was seen rostrally (Fig.2.8A-D). The coarse fibres in the most superficial layer extended further caudally than the finer deeper

fibres which became sparser gradually and disappeared close to the caudal pole (Fig.2.8F). On the ipsilateral side, much sparser label was seen superficially and rostrally (Fig.2.8B).

Distribution of retinal projection in the SC. A similar distribution pattern of label to that seen at 8 days was obtained contralaterally and ipsilaterally.

14-16 days (n=2)

Retinal fibre projection. The crossed optic fibres in the SC extended superficially towards the caudal pole. The thickness of both the coarse and fine label had increased from that seen at the previous age and the fibres gradually decreased in density caudally (Fig.2.9A-F). Fibres had still not reached the caudal pole. The fine label mainly seen at the rostral and lateral edges, deep to the most superficial layer, extended deeper into the SC than at earlier stages (Fig.2.9A-D). The uncrossed fibres, localized in the rostrolateral part of the ipsilateral SC, had increased compared to the previous age, but were still sparse compared to the contralateral projection (Fig.2.9A, B).

Distribution of retinal projection in the SC. By 16 days, the contralateral projection covered almost the entire rostral-caudal extent of the SC and extended further medially than at earlier ages to occupy approximately four fifths of the SC along the medial-lateral extent. Ipsilaterally, the retinal projection was confined to rostral and lateral SC (Fig.2.10).

18 days (n=1)

Retinal fibre projection. The nature of label in the contralateral and ipsilateral SC appeared unchanged from that seen at the previous stage.

Distribution of retinal projection in the SC. From this stage, in the SC on the side contralateral to the eye injection, optic axons first reached the caudal pole. They extended also more medially. Fibres distributed ipsilaterally still covered a small region in the lateral and rostral SC (Fig.2.11).

20-23 days (n=2)

Retinal fibre projection. The denser label was distributed in the most superficial layer across the whole surface of the SC, with label becoming coarser and fibrous in appearance at the caudal pole (Fig.2.12A-F). The label beneath this extended deeper into the SC, being seen as a distinctive narrow crescent-shaped region of HRP product in which the label was finer and less dense (Fig.2.12A-D). On the ipsilateral side, more labelled axons than those at earlier stages were seen superficially and for the first time finer label was seen to extend deep to the superficial label (Fig.2.12A, B).

Distribution of retinal projection in the SC. From this stage (Fig.2.13), the distribution of retinal axons labelled by HRP was more widespread in the contralateral SC, compared with that at younger ages. The axons invaded almost the entire SC, extending across approximately 90% of the medial-lateral and 100% of the rostral-caudal extent. The retinal projection on the ipsilateral side spread slightly more extensively, both in the lateromedial and rostrocaudal extent, compared to that seen at 18 days.

26-29 days (n=2)

Retinal fibre projection. At this stage, the nature of the labelled fibres projecting both contralaterally and ipsilaterally were similar to that seen at 20-23 days.

Distribution of retinal projection in the SC. From 26 days, the crossed projection first reached the medial border except at the most caudal pole. The uncrossed projection was distributed still at the rostral and lateral part of the ipsilateral SC (Fig.2.14).

35 days (n=1)

Retinal fibre projection. At this age, contralateral fibres deep to the most superficial layer were spread relatively evenly throughout the rostrocaudal and lateromedial extent (Fig.2.15A, B), with exception of the far caudal pole (Fig.2.15C). On the ipsilateral side, for the first time, a concentration of label was seen more deeply at the rostral pole and coarse fibres positioned most superficially became sparser (Fig.2.15A). Further caudally, finer fibres were seen in the superficial layer (Fig.2.15B). The ipsilateral label was densest rostrally and decreased towards the caudal pole (Fig.2.15A-C).

Distribution of retinal projection in the SC. Distribution of labelled fibres across the contralateral SC remained unchanged. Labelled axons ipsilateral to the injected eye were distributed much more extensively across the rostral-caudal and lateral-medial extent of the SC than at the previous age, but no fibres reached the medial border or caudal pole of the SC (Fig.2.16).

39 days (n=1)

Retinal fibre projection. Ipsilaterally, patches first appeared more deeply with the loss of label more superficially. This was only seen in the more rostral SC (Fig.2.17).

Distribution of retinal projection in the SC. The distribution of the retinal projection in the contralateral and ipsilateral SC was similar to that seen at the previous stage.

46 days (n=1)

Retinal fibre projection. Contralaterally, coarse label extended superficially over the entire SC. Finer label dispersed in the layer deep to this was seen from the rostrolateral to the caudomedial pole. Ipsilaterally, label in the superficial layer was sparse extending from rostral to caudal, while label concentrated in deeper layers was patchy falling off in density caudally (Fig.2.18). Up to and including this stage, the SC was not differentiated clearly into the cyto-architectural layers seen in adults (Wye-Dvorak, '84).

Distribution of retinal projection in the SC. Label in the superficial layer first extended throughout the entire rostrocaudal and lateromedial extent of the SC on both the contralateral and ipsilateral side (Fig.2.19).

Figure 2.1 Brightfield micrographs of unstained (A, C, E, F) and stained (B, D) coronal sections through the diencephalon and pretectum after an intraocular injection of HRP at 2 days

Contralateral to the injected eye is on the right. **(A)** and **(B)**: An unstained section and an adjacent stained section show that on the contralateral side, a few labelled axons indicated by arrows run parallel to the lateral surface of diencephalon. Bar: 200 μm. **(C)** and **(D)**: An unstained section and an adjacent stained section at the pretectal level shows that the fibre fascicles in the optic tract indicated by arrows reach the pretectum but do not achieve the rostral pole of the SC. Bar: 100 μm. **(E) and (F)** show high-power views of a few fine labelled axons, which are seen in (C) and (D), on the surface of the diencephalon laterally. Bars: 50 μm.

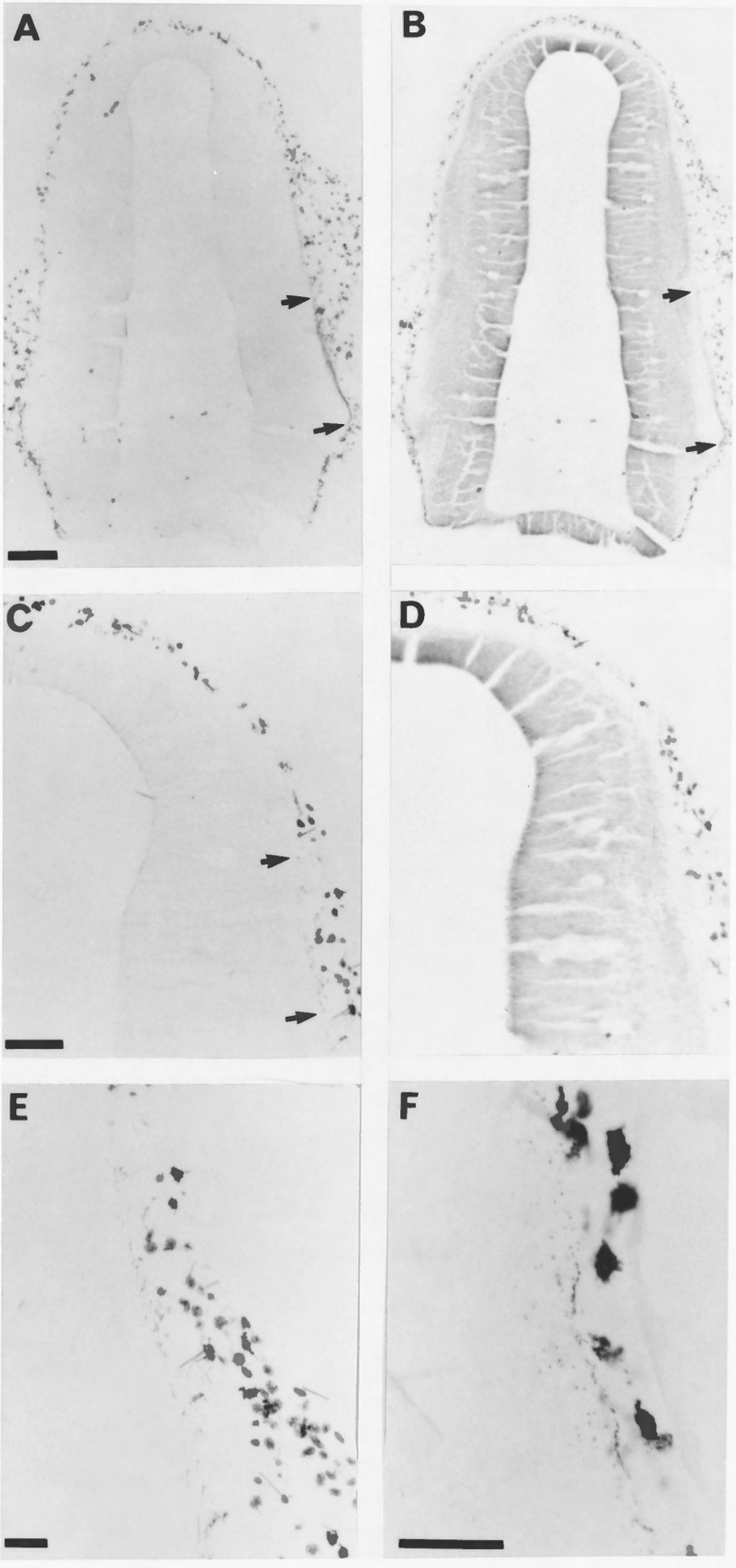
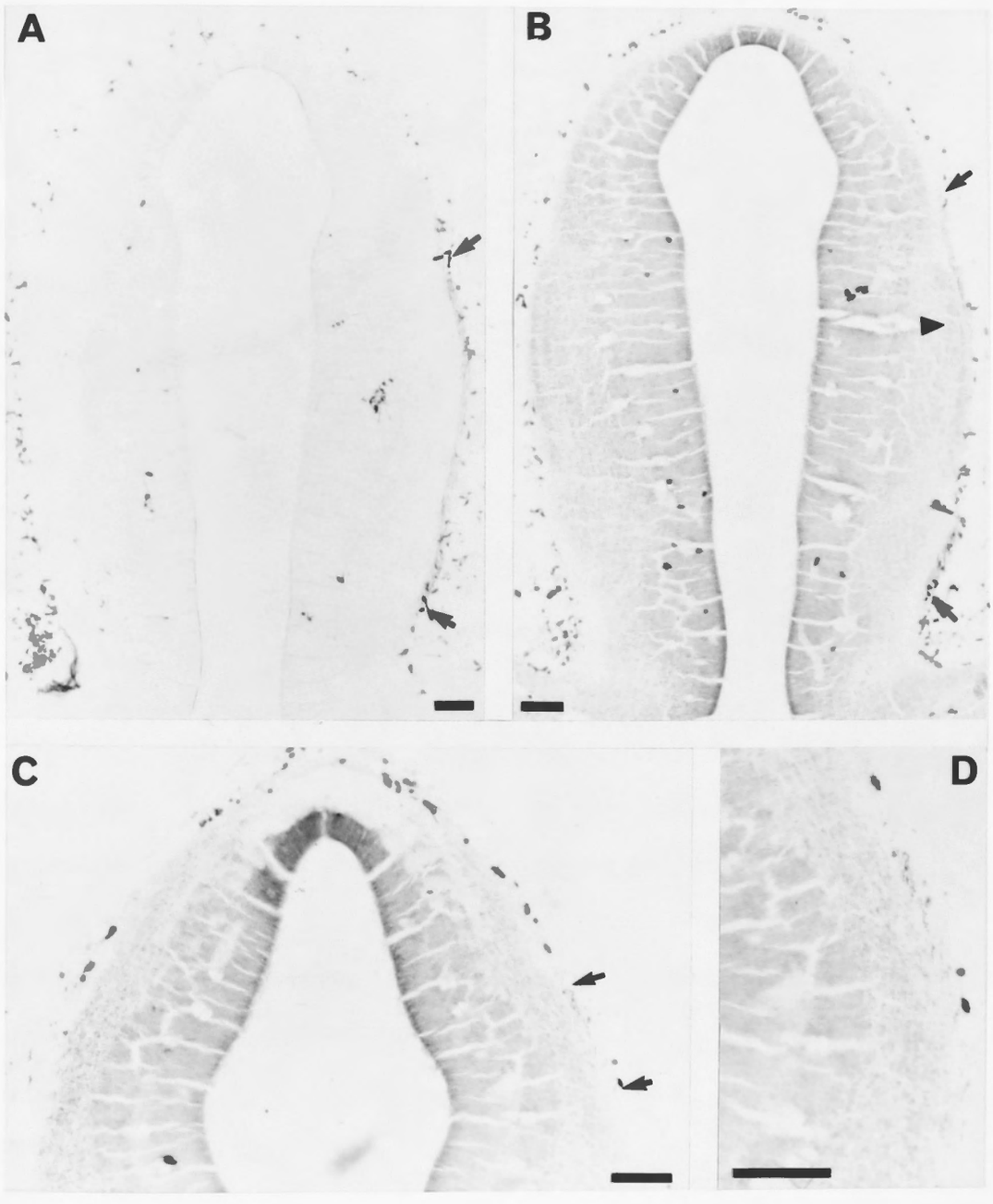

A
B
C
D
E
F

Figure 2.2 Brightfield micrographs of unstained (A) and stained (B, C, D) coronal sections through the diencephalon and SC after an intraocular injection of HRP at 4 days

Contralateral to the injected eye is on the right. **(A)** and **(B)**: An unstained section and an adjacent stained section show that many fine HRP labelled axons indicated by arrows are present along the contralateral optic tract and run over the surface of the lateral geniculate body (arrowhead). **(C)**: At the collicular level, label indicated by arrows is first visible in the rostral and lateral pole of the contralateral SC. **(D)**: A high-power view of the label seen in (C). Bars: 100 μm.


A
B
C
D

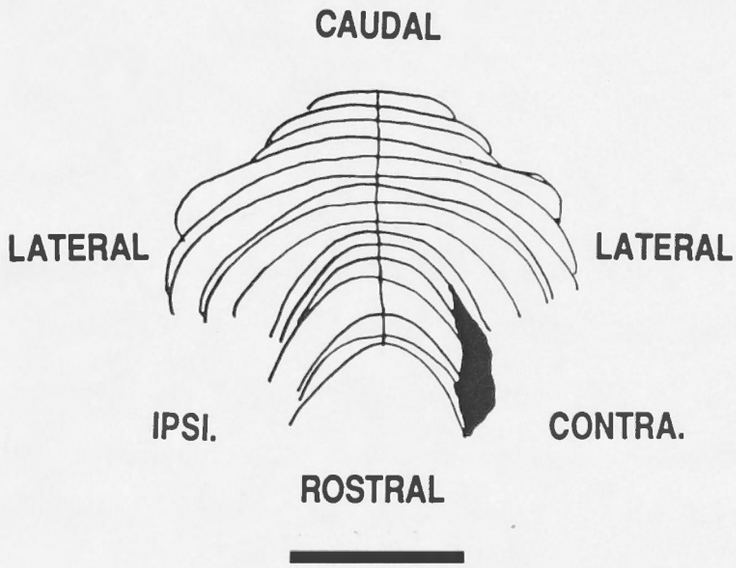
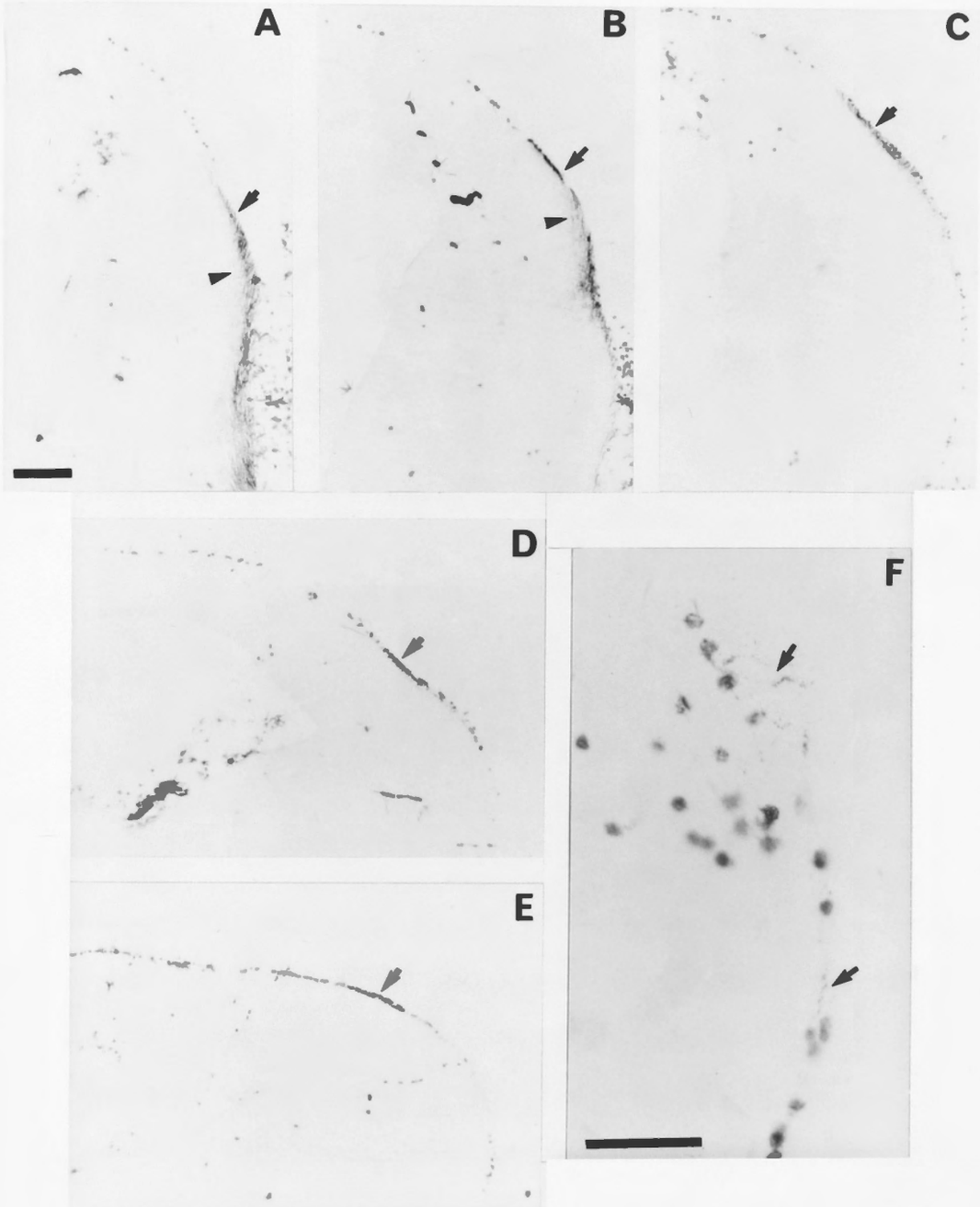


Figure 2.3 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 4 days

The black area indicates the retinal projection to the contralateral SC. The retinal axons are distributed rostrolaterally in the contralateral SC. No axons are seen in the ipsilateral SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

Figure 2.4 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 5 days

Contralateral to the injected eye is on the right. **(A-E)**: Sections shown run from the rostral pole of the SC (A) to close to the caudal pole (E) (see figure 2.5). Many labelled axons are distributed in the superficial layer of the contralateral SC (arrows). Axons extend rostrocaudally along the lateral aspects of the collicular surface just underneath the pia and decrease in density towards the caudal pole. They do not reach the caudal pole (see figure 2.5). Some fine HRP reaction product is first seen deep to the superficial axons laterally (arrowheads in A, B). Bar: 100 μm. **(F)** presents a high power view close to the far caudal pole showing the most caudal label. Only a few individual axons indicated by arrows are seen. Bar: 50 μm.


A
B
C
D
E
F

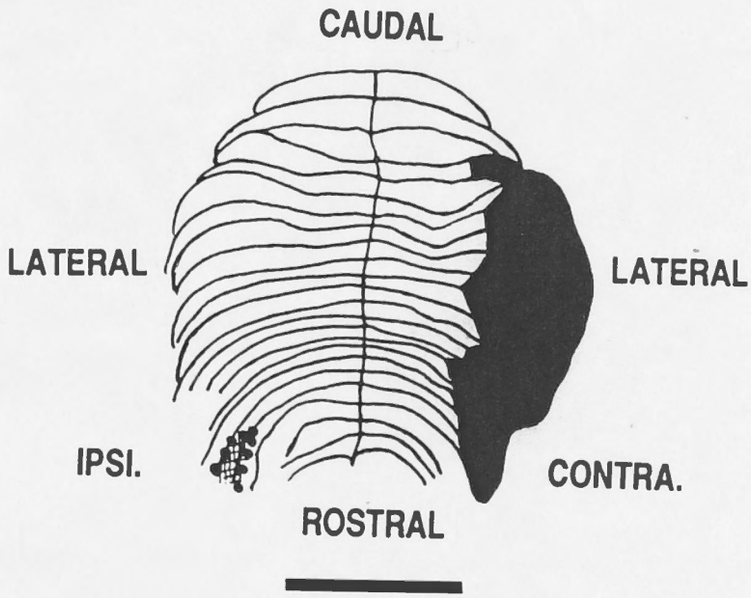


Figure 2.5 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 5 days

Conventions are the same as for figure 2.3. In addition, the cross hatching indicates the area of the retinal projection to the ipsilateral SC. At this stage, labelled fibres contralaterally occupy the lateral half of the SC and extend over most of the rostral-caudal extent, with the exception of the caudal pole. For the first time, fibres begin to invade the rostral and lateral pole of the ipsilateral SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

Figure 2.6 Brightfield micrograph of unstained coronal section through the rostral SC after an intraocular injection of HRP at 8 days

Labelled axons cover the contralateral SC laterally. The label in the superficial layer has become thicker and the finer label (arrow) extended deep to this has increased in depth, compared with that seen at the previous age. Bar: 100 μm.

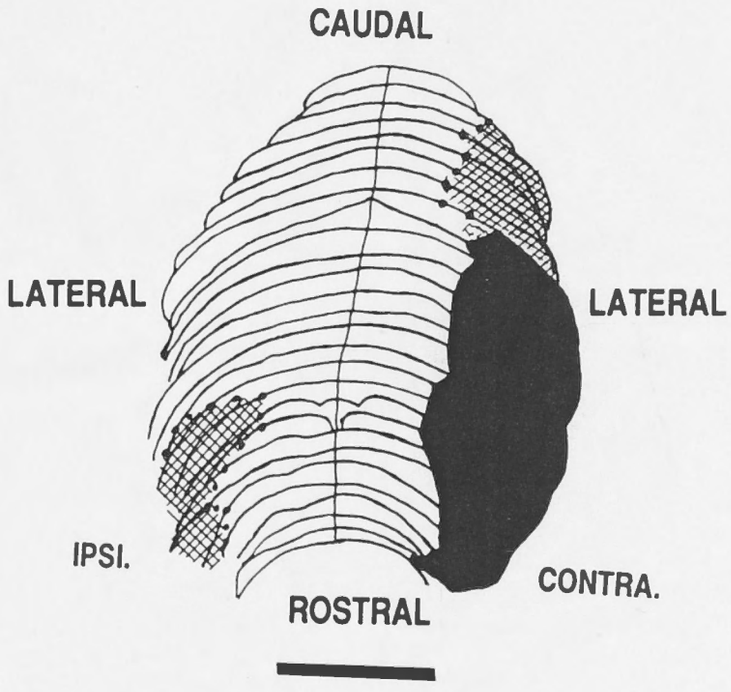


Figure 2.7 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 8 days

Conventions are the same as for figure 2.3 and 2.5. At this stage, the size of the SC increases in rostral-caudal and medial-lateral extent. Labelled axons in the contralateral SC are distributed laterally in rostrocaudal extent, similar to that seen at the previous stage. Fibres extend further caudally and become sparser at the caudal pole (indicated by cross hatching). Ipsilateral axons are seen in the SC rostrolaterally. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

Figure 2.8 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 10 days

Contralateral to the injected eye is on the right. **(A-E)**: Sections shown run from the rostral pole of the SC (A) to close to the caudal pole (E). Coarse retinal fibres in the rostral pole of the contralateral SC are prominent superficially and spread caudally with decreasing density. They do not reach the caudal pole. On the ipsilateral side, much sparser label indicated by arrows is distributed superficially at rostral pole of the SC (B). Finer HRP reaction product is seen contralaterally underneath the coarse fibres (A-D), being absent further caudally (E). Bar: 100 μm. **(F)** shows a high power view demonstrating that the most caudal label in the superficial layer (arrows) is visible close to the caudal pole of the contralateral SC. Bar: 50 μm.

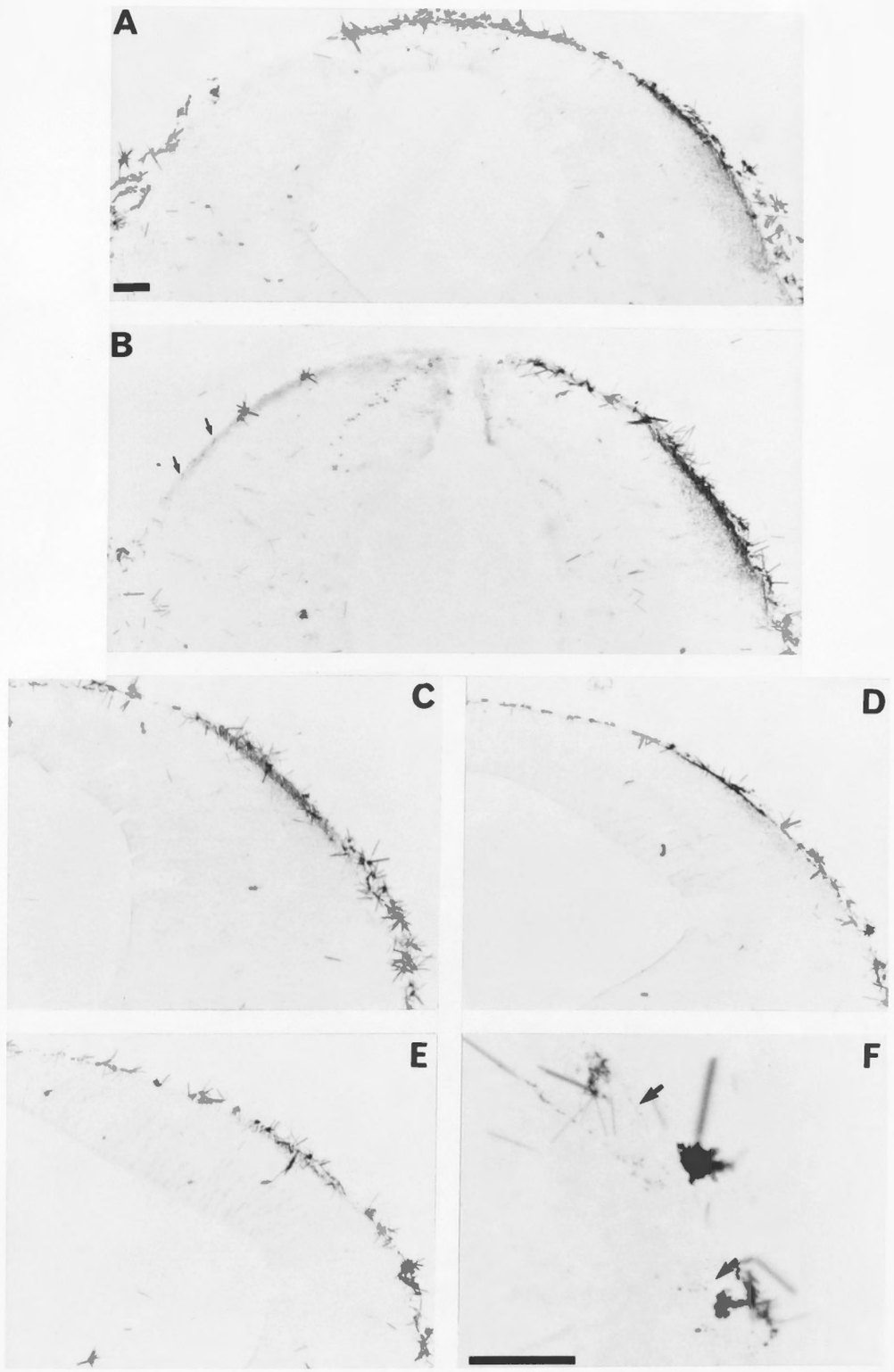
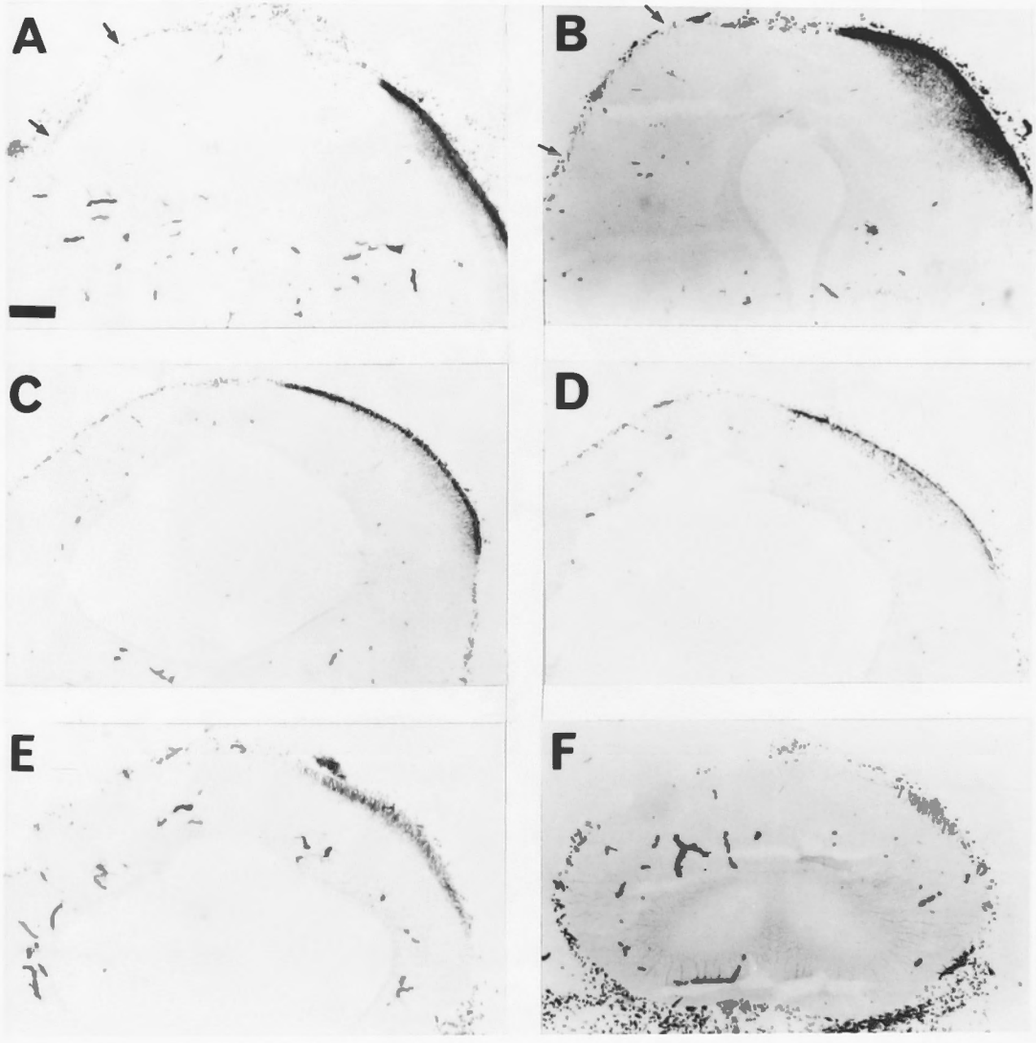

A
B
C
D
E
F

Figure 2.9 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 14 days

Contralateral to the injected eye is on the right. **(A-F)**: Sections shown run from the rostral pole of the SC (A) to close to the caudal pole (F). The heavily labelled optic fibres in the rostral pole of the contralateral SC extend caudally. The fibres gradually decrease in density towards the caudal pole. The fine label deep to this is seen mainly in the rostral-lateral portion and increases in depth compared with that seen at the previous stage (A-D). Much sparser fibres than those seen contralaterally, indicated by arrows, are localized superficially in the rostrolateral part of the ipsilateral SC (A, B). Bar: 100 μm.


A
B
C
D
E
F

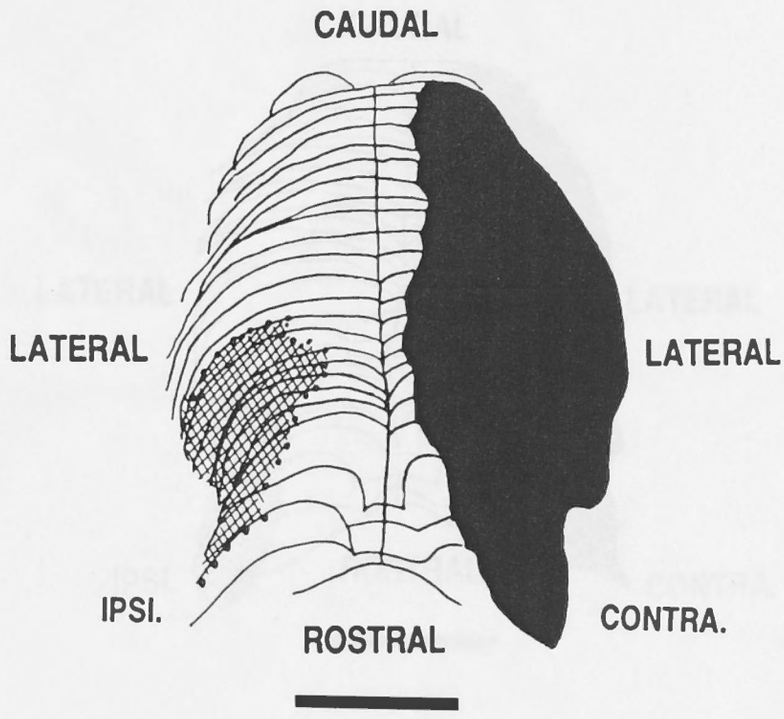


Figure 2.10 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 16 days

Conventions are the same as for figure 2.3 and 2.5. The three dimensional reconstruction shows that the retinal projection covers contralaterally almost the entire rostral-caudal extent of the SC and four fifth of the SC along the medial-lateral extent. Ipsilaterally, the retinal projection, confined to the rostrolateral part of the SC, is distributed slightly more extensively, compared with that seen at younger stages. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

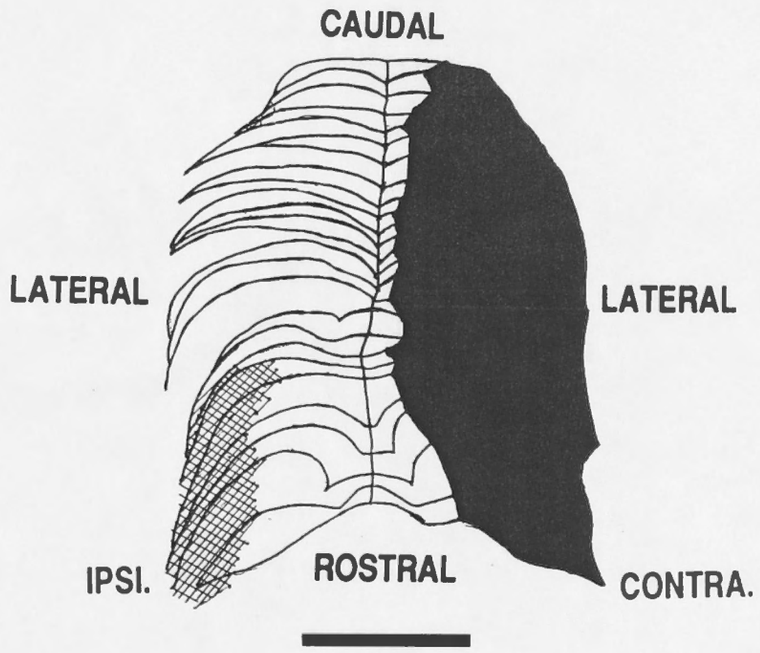
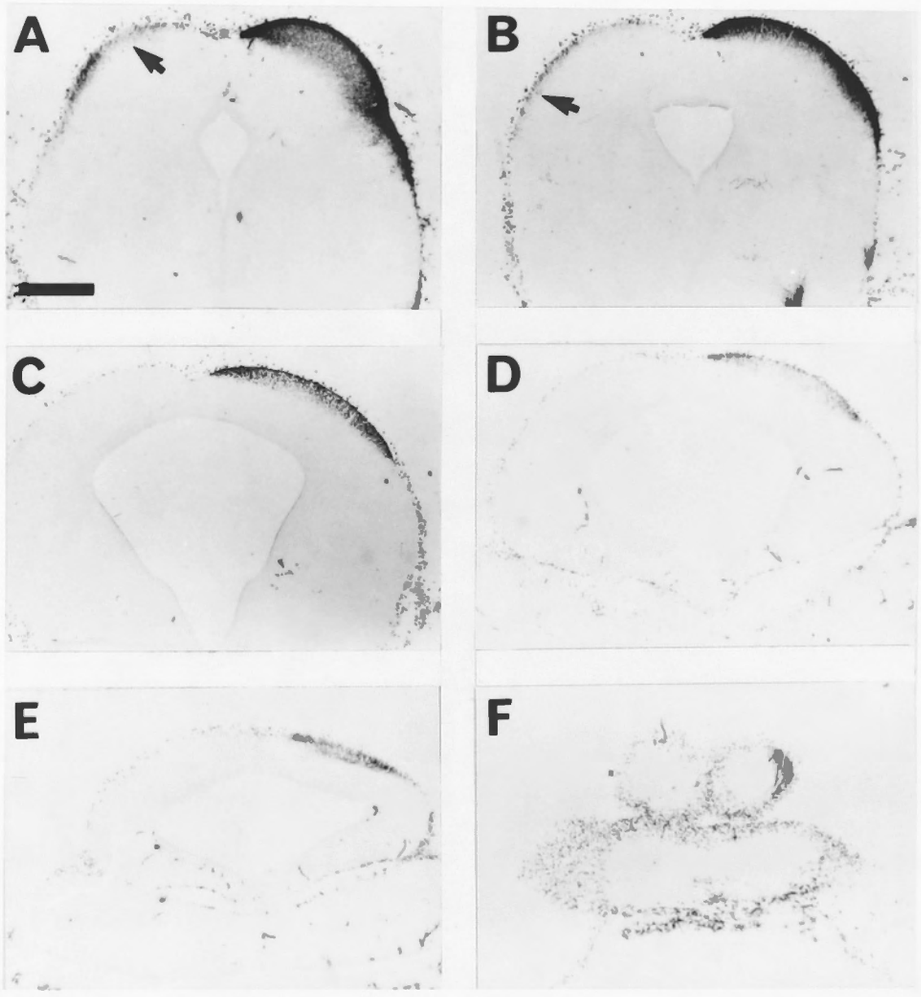


Figure 2.11 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 18 days

Conventions are the same as for figure 2.3 and 2.5. At this stage, optic axons in the SC on the side contralateral to the injected eye reach the caudal pole for the first time. They also extend more medially. Fibres distributed ipsilaterally are confined in the lateral and rostral SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

Figure 2.12 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 20 days

Contralateral to the injected eye is on the right. **(A-F)**: Sections are at intervals through the SC. The rostral pole of the SC is shown in (A) and the caudal pole is shown in (F). Denser fibres growing from the optic tract are distributed in the outermost superficial layer, across almost the entire surface of the SC except for the most medial edge. Label becomes coarser and fibrous in appearance more caudally (C-F). The finer label deep to this increases in depth more rostrally, compared to that seen at earlier stages, being restricted in a distinctive narrow crescent-shaped region (A, B). On the ipsilateral side, more axons, compared with those seen at the previous stage, are seen rostrally (A, B). Finer label indicated by arrows is detected deep to this for the first time (A, B). Bar: 500 μm.


A
B
C
D
E
F

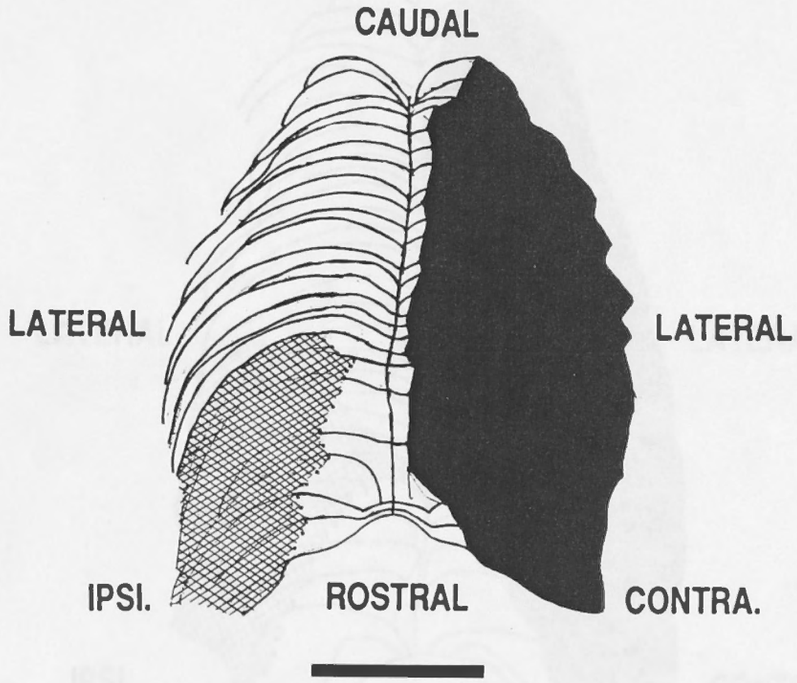


Figure 2.13 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 20 days

Conventions are the same as for figure 2.3 and 2.5. Optic axons in the contralateral SC extend further medially, compared with that seen at earlier stages. Ipsilaterally, fibres are distributed slightly more extensively in the lateral and rostral SC, compared with that seen at the previous age. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

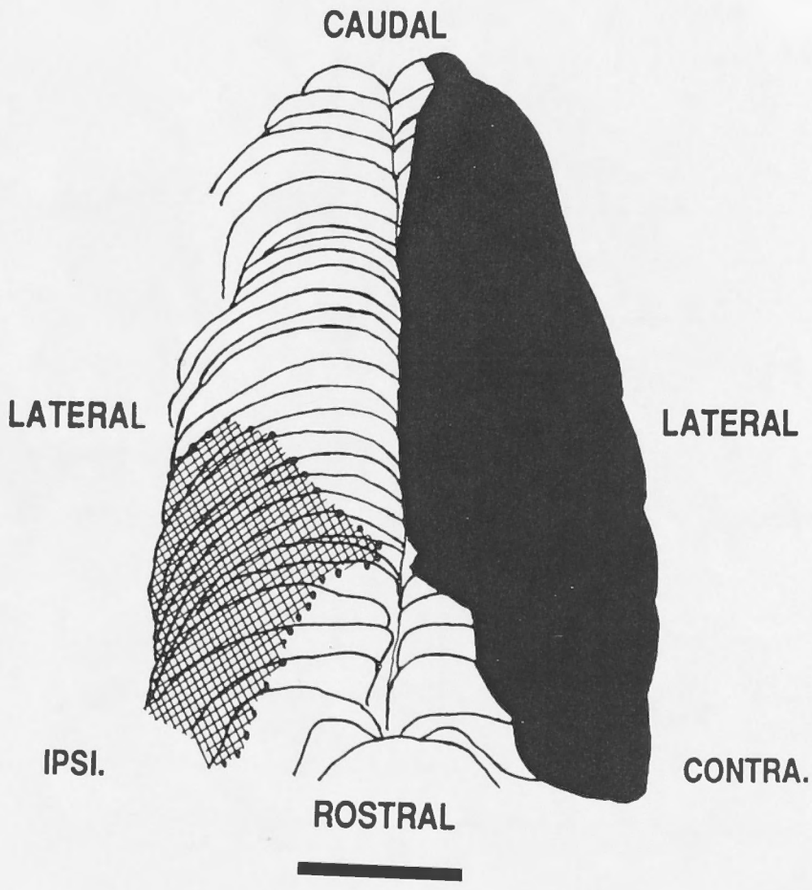
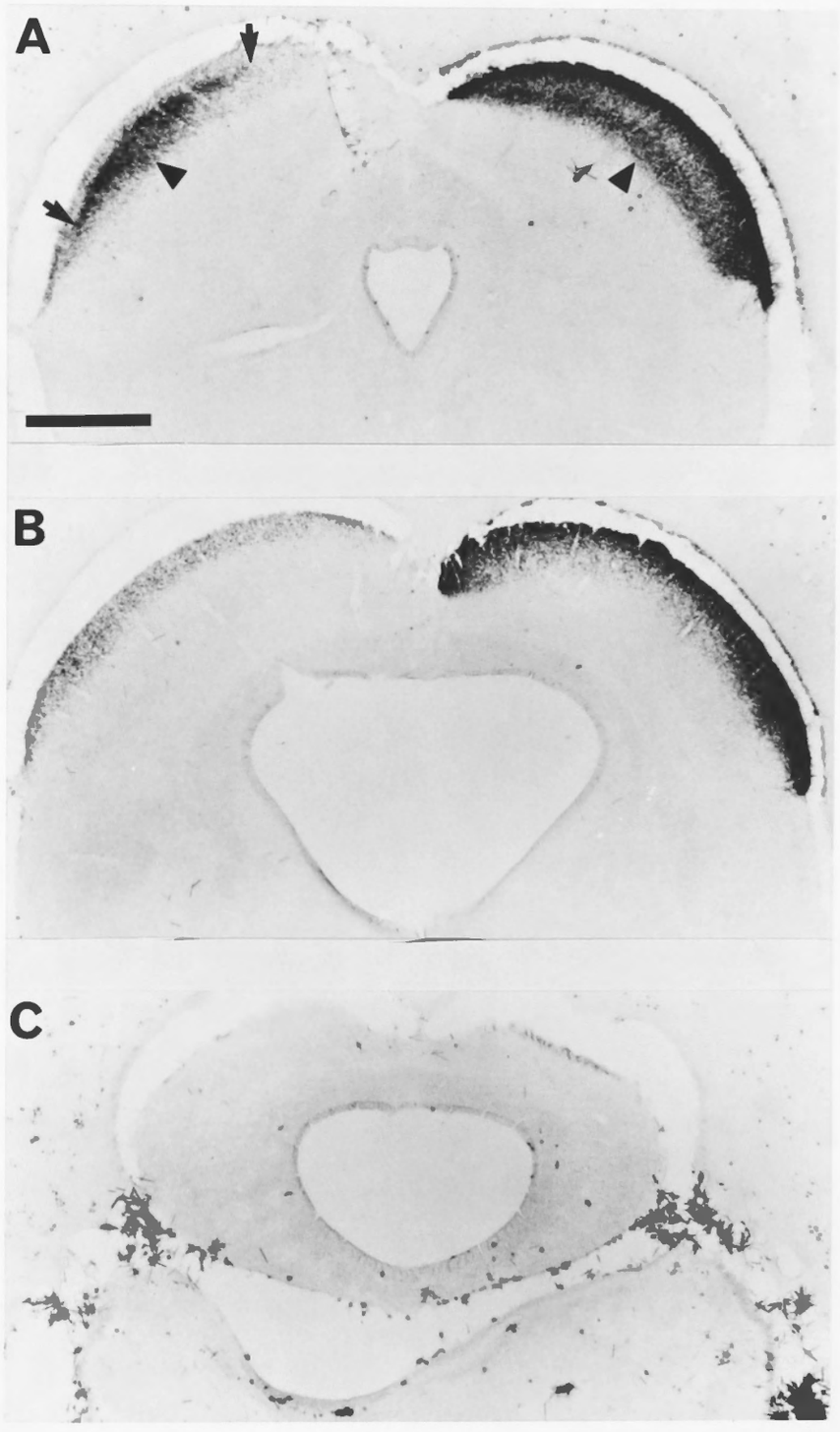


Figure 2.14 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 26 days

Conventions are the same as for figure 2.3 and 2.5. For the first time, the crossed projection extends to the medial border except for the far caudal pole from this stage. Uncrossed fibres are spread in the rostral and lateral part of the SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

Figure 2.15 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 35 days

Contralateral to the injected eye is on the right. **(A-C)**: Sections shown run from the rostral pole of the SC (A), futher caudally (B) to the caudal pole (C). Retinal fibres are labelled heavily in the outermost layer throughout the contralateral SC. Lighter label deep to this spreads over the rostrocaudal and mediolateral extent (A, B) except for the caudal pole (C). On the ipsilateral side, retinal fibres projecting to the rostral SC begin to concentrate in the deeper layer (arrows) and the fibres positioned superficially become sparser (A). Further caudally, fibres are found evenly distributed in the superficial layer (B). Arrowheads in (A) indicate label in the pretectal area on both sides. Bar: 500 μm.


A
B
C

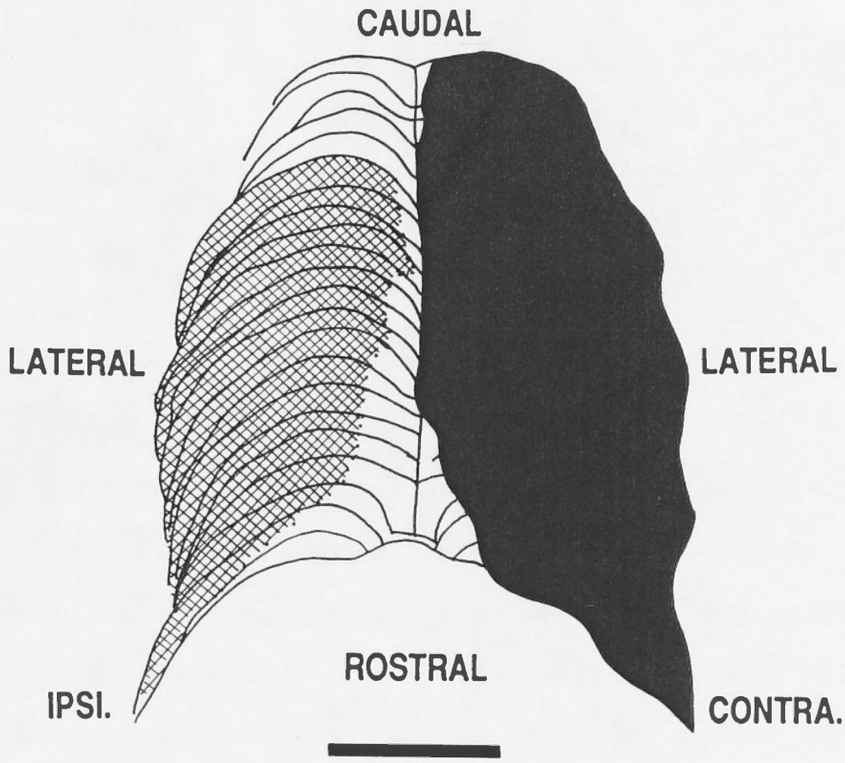


Figure 2.16 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 35 days

Labelled fibres contralateral to the injected eye are distributed across almost the whole surface of the SC. Ipsilaterally, the distribution of labelled axons is more extensive than at the previous age, being scattered extensively in the rostral and lateral SC, but no fibres reach the medial border or caudal pole of the SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

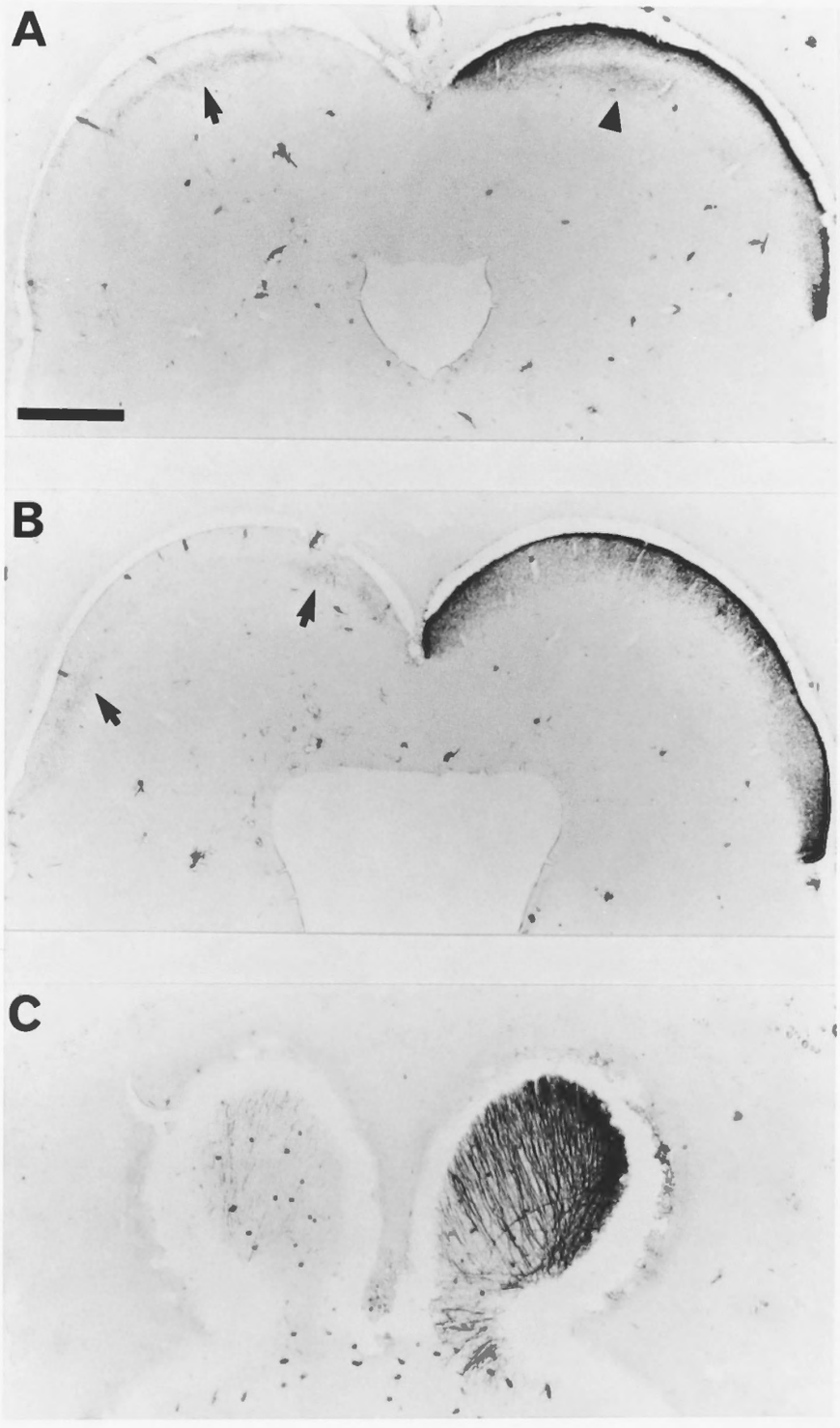
Figure 2.17 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 39 days

Contralateral to the injected eye is on the right. Two examples **(A)** and **(B)** of the more rostral regions are shown here. On the contralateral side, the coarse label is positioned superficially and finer label, distributed evenly, is seen deep to this (A, B). On the ipsilateral side, for the first time, the retinal projection to the more rostral SC is distributed mainly underneath the outermost layer, with the beginning of the appearance of patches (arrows in A). More caudally, the label is still distributed superficially (B). Bar: 500 μm.


A
B

Figure 2.18 Brightfield micrographs of unstained coronal sections through the SC after an intraocular injection of HRP at 46 days

Contralateral to the injected eye is on the right. **(A-C):** The rostral pole of the SC is shown in (A), futher caudal in (B) and the caudal pole in (C). Contralaterally, heavy label in the more superficial layer extends over the entire SC. Finer fibres concentrated deep to this layer are seen throughout the SC from rostrolateral to caudomedial pole. Ipsilaterally, labelled axons extend rostrocaudally, reaching the caudal pole for the first time (C). The label is mainly deeper and patchy (arrows in A, B). Arrowhead in (A) indicates label in the contralateral pretectum. Bar: 500 μm.


A
B
C

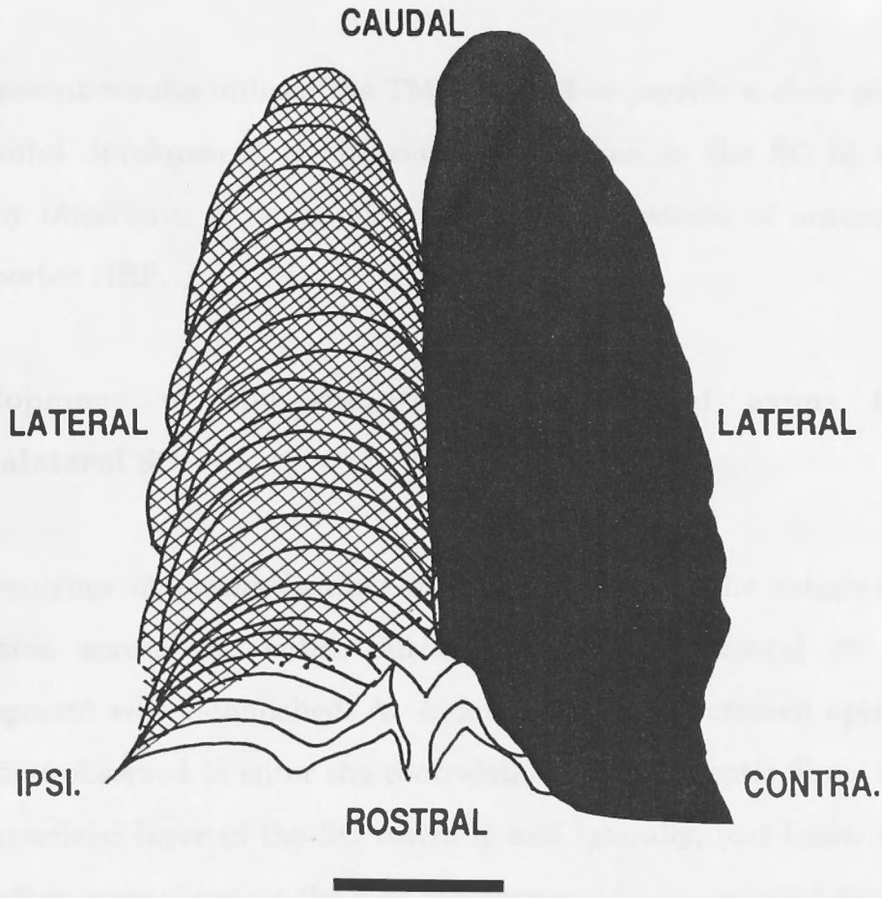


Figure 2.19 Map reconstructed from camera lucida drawings of coronal sections through the SC after an intraocular injection of HRP at 46 days

For the first time, labelled axons are seen to distribute both contralaterally and ipsilaterally throughout the entire rostrocaudal and mediolateral extent of the SC. CONTRA: contralateral; IPSI: ipsilateral. Bar: 1 mm.

DISCUSSION

The present results utilized the TMB method to provide a clear picture of the initial development of the retinal projection to the SC in tammar wallaby *(Macropus eugenii)*, by revealing the presence of anterogradely transported HRP.

Development of the distribution of retinal axons in the contralateral SC

The sequence of events leading to the formation of the retinocollicular projection across the entire surface of the contralateral SC during development was established. At 4 days after birth, crossed optic fibres were first observed to enter the contralateral SC. The optic fibres invaded the superficial layer of the SC rostrally and laterally, just below the pia. Thereafter, projections on the side contralateral to the injected eye tended to extend caudally and medially. They became widespread gradually throughout the rostral to caudal and lateral to medial extent of the SC. Axons reached the caudal pole by 18 days. From 20 days, the retinal projections extended more widely from the lateral to medial SC and finally reached the medial border along most of the rostro-caudal extent except for the far caudal pole by 26 days. Retinal axons extended to the medial border at the far caudal pole by 46 days.

The depth distribution of the retinocollicular projection was also demonstrated contralaterally. From 5 days soon after retinal axons entered the SC, the labelled axons were detected to pass inwardly into the deeper layer. This event was first seen rostrally and laterally. At subsequent ages from 8 to 29 days, the thickness and extent of label in the deeper layer

increased gradually, although more label was seen at the lateral rather than at the medial aspect of the SC. By 35 days, label was distributed relatively evenly throughout the whole depth of the deeper layer throughout the entire SC.

Thus, it is clear that from the initial stages, the retinocollicular projection proceeds from the rostrolateral pole of the SC where the fibres from the optic tract begin to enter. The projection extends gradually in three dimensions: caudally, medially and deeply. Sequentially, this projection reaches the caudal pole first, followed by the medial edge and then distributes throughout the deeper layer.

In the present study, the coarse label was seen in the most superficial layer of the SC while finer label was observed deep to this layer. The coarse label here may represent bundled axons on the surface of the SC and finer label may represent the individual axons as they extended deeply. This finding does not suggest that the finer label represent the arborization of retinal axons in terminals because no evidence of terminals is found until after these ages (see chapter 4).

The development of the retinocollicular projection on the ipsilateral side

Development of the retinal projection to the ipsilateral SC occurred later than that seen on the other side. HRP label appeared a day later in the ipsilateral SC with axons invading the rostrolateral pole, as they do contralaterally, at 5 days. Gradually, the projection was distributed extensively in the rostrolateral part of the SC up to 26-29 days and by 35 days the retinal axons innervated further caudally and medially. At 46

days when the retinal projection occupied the entire contralateral SC, label also covered the entire ipsilateral SC as well. The projection invaded the deeper layer later than that on the contralateral side. Fibres in the superficial layer invaded the deeper layer rostrally and laterally at 20 days, rather than at 5 days as found contralaterally. By 35 days, label in the rostral SC became sparser superficially. From 39 days, the first appearance of patches was present rostrally. At 46 days, the ipsilateral label was confined clearly to a thin, patchy strip lying just beneath the superficial layer. At all stages in the present study, the label in the ipsilateral SC was lighter than on the other side in both superficial and deeper layers. Other studies have shown that the patchy ipsilateral label concentrated in deeper layers began to fall off caudally in density at 56 days (Marotte, '90) and label was absent from the caudal third of the ipsilateral SC at 72 days, when the pattern was similar to the adult (Wye-Dvorak, '84).

The consideration of techniques between the autoradiography and the HRP

A different time course of establishment of the retinal projection to the SC was observed in another study in the tammar wallaby *(Macropus eugenii)* (Wye-Dvorak, '84), using autoradiography after injections of 3H-proline into one eye to trace the projection. Although label could be seen over the optic chiasm coursing toward visual areas in the contralateral thalamus at 4 to 5 days, it was not until 12 to 14 days that label could be seen over the ventrolateral surface of the contralateral SC, and not until 21 to 22 days that light label was seen over the superficial layer of the ipsilateral colliculus. The label concentrated in the deeper layer of the ipsilateral SC appeared patchy at 63-64 days. In the present experiment with HRP

technique, retinal axons entered the superficial layer of the contralateral SC at 4 days, that is 8 to 10 days earlier than that previously reported. Axons reached the ipsilateral SC at 5 days, that is 16 to 17 days earlier than that observed autoradiographically. The ipsilateral label lying beneath the superficial layer became patchy at 39 days, that is 14-15 days earlier than that seen autoradiographically. The different results obtained by the autoradiographic technique and the anterograde tracing technique with HRP support the comment that there is a limitation in the autoradiographic method, because it is difficult to detect very sparse projections above background levels of label at early developmental ages (Frost et al., '79; Jen et al., '84). This is not a problem with HRP as the genuine anterograde label can be recognized unequivocally.

Comparison with other mammals

An essentially similar sequence of development of the retincollicular projection to the contralateral SC, to that seen in the wallaby, was found in rodents. As in the wallaby, the first optic axons to reach the rostral border of the SC were seen early in development, followed the next day by axons growing across the entire rostrocaudal extent with the exception of the medial and lateral edges in rat (Lund and Bunt, '76). A lighter projection was also detected in the ipsilateral SC, a day later than that seen contralaterally, and at this time the projection both contralaterally and ipsilaterally covered the entire SC (Bunt et al., '83). The sequence found in mouse (Godement et al., '84; Edwards, '86), was also similar to that seen in the wallaby. The first invading fibres from the retina were detected rostrally in the contralateral SC and the labelled fibres ran in the rostrocaudal direction across the surface of the SC underneath the pia later on. Ipsilaterally, rare single fibres first entered the SC rostrally a few days

later than the other side and then grew rostrocaudally over the surface of the SC. The mature pattern of the ipsilateral projection, in which retinal fibres are concentrated in the rostral part of the SC, was seen later on. In hamster, observations made on the day of birth indicated that labelling extends over about 60% of the distance from the rostral to caudal pole of the contralateral SC. At 3 days after birth, the entire extent of the contralateral SC was covered by labelled retinal axons. At all developmental stages after birth, uncrossed retinotectal projections were seen to be restricted to the rostral half of the SC as seen in the adult (Frost et al., '79). However, sparsely distributed fibres were found ipsilaterally to cover the entire area and depth of the superficial layers using a similar tracing technique with HRP in adult hamster (Jen et al., '84). Unlike the sequence, the timing of the development in the rodents is different from that seen in the wallaby. The time from the day when fibres enter the SC to the day when fibres cover the entire SC extends over only several days in the rodents, whereas the same process is completed in several weeks in the wallaby.

Although the initial development of the retinocollicular projection is not available in some studies as the retinal axons had been found to cover most of or the entire SC contralaterally at the earliest time examined, the subsequent events in the development of the retinal projection to the ipsilateral SC have been described in the studies. These are similar to that seen in the wallaby. In the marsupial opossum (Cavalcante and Rocha-Miranda, '78), earliest evidence for fibres to the ipsilateral SC was found to be restricted, being absent from the extreme rostromedial and caudolateral portion initially, followed by the whole rostrocaudal extent of ipsilateral projection being established. Finally, the ipsilateral projection was distributed in the rostral half of the SC. However, in the quokka wallaby

(Harman and Beazley, '86), the retinal projection to the ipsilateral SC was seen to be confined to the rostral half of the SC at all developmental stages. This study used an anterograde transport of a tritiated amino acid followed by autoradiography which is less sensitive than HRP. Therefore, the sparse projection to the more caudal SC seen in other species may not have been detected. In other studies, although the extent of this projection varied from one species to another, it was consistently found in the species studied that ipsilateral input to the SC is widespread across the entire SC at a stage early in development but later becomes restricted rostrally as in the mature animals. This pattern was reported in rat (Land and Lund, '75), cat (Williams and Chalupa, '82), grey squirrel (Cusick and Kaas, '82) and rabbit (Crabtree, '89), although this projection was found to be largely excluded from the extremely rostral pole of the SC in the cat (Williams and Chalupa, '82).

Early development of the retinotectal projection in non-mammalian vertebrates

A similar pattern to that shown in the wallaby, in which retinal axons grew in a curvilinear fashion from the rostrolateral pole of the SC where the optic tract fibres entered, to their caudomedial pole during development, was also reported in non-mammalian vertebrates such as frog (Gaze et al., '74; Currie and Cowan, '75; Holt, '84), fish (Stuermer, '88a) and chick (De Long and Coulombre, '65; Goldberg, '74; Crossland et al., '75; Rager and von Oeynhusen, '79; Thanos and Bonhoeffer, '83; Fujisawa et al., '84; McLoon, '85).

The timing and sequence of initial retinotectal projections has been studied eletrophysiologically and anatomically in frog. The earliest visual

responses were recorded in the optic tectum from the stage when the first signs of the appearance of a recognizable tectal structure occur in *Xenupus.* The responses were only obtained from the front half of the tectum, while the caudal part of the tectum was unresponsive early in development (Gaze et al., '74). Anatomically, also in *Xenopus*, optic axons grow into the tectum in a sequence, in which the rostral tectum is first innervated and the more caudal part begins to be innervated later on (Holt, '84). In addition, results in *Rana* indicated that retinal ganglion cell axons first reaching the optic tectum are confined to the rostrolateral portion of the tectum. Progressively, the retinal afferents extended over the tectum in a rostroventrolateral to caudodorsomedial sequence. This innervation pattern of retinal axons to the tectum was found to roughly match the caudomedial gradient of cell proliferation and cytoarchitectonic differentiation (Currie and Cowan, '75). An earlier study (Kollross, '53) also demonstrated a rostrocaudal sequence of tectal maturation and cell addition in *Rana.* Later work also showed tectal histogenesis in a regular rostrolateral to caudomedial sequence, in which the tectal cells are added at the caudomedial margin of the tectum, in *Xenopus* and *Rana* (Straznicky and Gaze, '72; Jacobson, '77; Gaze et al., '79). In another marsupial wallaby, the quokka, although a rough rostrocaudal gradient of cell addition occurred, it is much less marked than in these frogs.

The sequence in which the initial retinal axons grow from the eye to the tectum has been reported in embryonic fish (Stuermer, '88a). A rostral to caudal sequence of ingrowth of the retinotectal projection was also seen. The first axons invaded the tectum rostrally. Within 8-10 hours postfertilization, many more axons grow into the tectum and covered the rostral half. Axons then gradually proceeded into the caudal tectal region over the next 12 hours.

In addition, similar events in the development of the chick retinotectal projection to that seen in the wallaby have been obtained. Development of the projection was demonstrated for the first time in the study of De Long and Coulombre ('65). Retinal fibres entered the tectum at its rostroventral margin initially, in embryonic development. This was followed by a steady progression of fibres expanding caudally and in a short time the surface of the optic tectum was covered completely by optic fibres. This pattern of invasion of the retinal projection was confirmed as determined with similar reduced silver techniques (Goldberg, '74) and with anterograde transport techniques (Crossland et al., '75; Thanos and Bonhoeffer, '83; Fujisawa et al., '84; McLoon, '85). A similar polarity of cell genesis and maturation in the tectum was also documented in chick, in which the rostral pole is the earliest to develop and the caudal pole is the last to develop (LaVail and Cowan, '71a, b; Rager, '80b). As in the wallaby, soon after the first retinal fibres were detected entering the tectum in chick, they were found penetrating from the surface into deeper tectal layers (Rager and von Oeynhausen, '79; McLoon, '85). However, in a study with autoradiographic techniques (Crossland et al., '75), the first retinal axons were seen to delay penetration below the superficial layer for 2 days after entering the tectum. This delay could be explained by differences in technique. In young embryo chick, the high background of autoradiographs may obscure smaller projections (McLoon, '85).

SUMMARY

The timing and sequence of events leading to the formation of the retinocollicular projection across the entire surface of the SC has been

established. From 4 days after birth as the first retinal axons enter the contralateral SC rostrolaterally, they gradually innervate the surface of the SC in a caudomedial sequence. Retinal axons begin to invade the rostrolateral portion of the ipsilateral SC a day later and they extend caudomedially as they do contralaterally. By 46 days, the entire SC both on the contralateral and ipsilateral side is covered by the retinal projections. How the outgrowth of ganglion cells from each quadrant of the retina correlates with this sequence in the retinal innervation to the SC during development becomes a focus in the next study.

Chapter 3. The Sequence Of Axon Outgrowth From The Developing Retina To The Superior Colliculus

INTRODUCTION

During early development of the retinal projection to the superior colliculus (SC) in the wallaby (chapter 2), the first axons from the retina reach the contralateral SC at the rostrolateral border by 4 days, and subsequently they spread caudally and medially over the SC. Initial axons reach the rostrolateral edge of the ipsilateral SC later on, within 24 hours. They then spread over the SC also in a caudomedial direction and cover the rostrolateral half of the SC by 16 days. The retinal projection covers the whole SC contralaterally and ipsilaterally by 46 days, with the patchy distribution characteristic of the adult ipsilateral projection (Wye-Dvorak, '84) beginning to appear in the ipsilateral SC. However, which part of the retina projects to the SC first and the subsequent order of outgrowth from different parts of the retina remain unknown. This sequence of retinal axon outgrowth and the distribution of retinal ganglion cells projecting contralaterally or ipsilaterally is significant for the mechanisms by which the topography of retinal projections is established.

The pattern by which retinal ganglion cells innervate their central target has been extensively examined in mammals. Studies on adult rodents such as rat and mouse demonstrated a projection pattern of ganglion cells, in which the crossed projection arises from the entire contralateral retina while the uncrossed projection arises from the ganglion cells restricted to a crescent-shaped area bordering the ipsilateral temporoventral retina

(Lund, '65; Cowey and Perry, '79; Drager and Olsen, '80; Jeffery et al., '81; Drager and Hofbauer, '84; Reese and Cowey, '86). Early in development, a similar plan to that seen in the adult was also demonstrated in developing mammals. During development, retinal ganglion cells projecting contralaterally and ipsilaterally are similarly distributed in rodents (Bunt et al., '83; Martin et al., '83; Insausti et al., '84; Jeffery, '84; Drager, '85; Sretavan, '90; Yhip and Kirby, '90), ferret (Henderson et al., '88; Cucchiaro, '91; Thompson and Morgan, '93) and cat (Williams et al., '86; Lia et al., '87). The contralaterally projecting ganglion cells are distributed across the entire retinal area, while the majority of the ipsilaterally projecting cells are restricted to the temporal crescent-shaped region but there are also some cells scattered across the rest of the retina. Subsequently, those ganglion cells outside the crescent-shaped region are preferentially eliminated.

However, little information is available on the initial development in outgrowth of ganglion cells from the retina to the SC at early stages when retinal ganglion cells first innervate the SC. A few studies on the sequence of retinal ganglion cell axon outgrowth initially in development have been carried out in rodents. Evidence that temporal retina is preferentially represented in the early retinocollicular projection was demonstrated in hamster (Wikler et al., '85), where in embryonic development axon outgrowth from the temporal portion of the retinal ganglion cell layer was detected contralaterally prior to that in nasal retina. Simultaneously, the ipsilateral projection arises from the temporal periphery of retina. In the mouse (Godement et al., '87; Colello and Guillery, '90), retinal ganglion cells projecting contralaterally are present first in the central area around the optic disc and later extend over the whole retinal area except for the most peripheral margin. It was also shown in these studies that the

earliest ipsilaterally projecting ganglion cells are located in the dorsal central retina, followed soon after by the appearance of an adult-like pattern, with the majority of ganglion cells being in the temporoventral crescent.

The aim of the present study is to define the sequence of retinal ganglion cell outgrowth from retina to the SC and the distribution of ganglion cells projecting to the SC contralaterally and ipsilaterally in wallaby by using the fluorescent lipophilic carbocyanine dye 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) as a retrograde tracer *in vitro*. This enabled accurate and extensive placement of crystals in very young animals, which would have been impossible *in vivo*. Tracer was placed across the rostral pole of one lobe of the SC to label all ganglion cell axons projecting from the contralateral and ipsilateral retina to this target. Pouch young aged from 4 days, when the first retinal axons reach the edge of the rostrolateral SC, to 46 days, when the retinal projection covers the whole surface of the contralateral and ipsilateral SC (chapter 2), were utilized.

MATERIALS AND METHODS

Animals

Forty seven pouch young wallabies (*Macropus eugenii*) were obtained from the RSBS wallaby colony at the ANU campus. Thirty one animals aged 4-5 (n=3), 8-9 (n=5), 11-13 (n=6), 16 (n=6), 28-36 (n=6) and 41-46 (n=5) days, were used for DiI labelling. Sixteen animals of similar ages ranging from 5 (n=1), 9 (n=1), 13-15 (n=4), 17-18 (n=3), 26-32 (n=6) and 45 (n=1) days,e were used to observe the distribution of the ganglion cell layer by using cresyl fast violet (CFV) staining. Animals under 28 days were of exactly

known birth date. Pouches were checked daily, usually in the morning. Ages of older animals were determined from a chart of head lengths of animals of known age (W.E. Poole, personal communication).

DiI placement

Pouch young were sacrificed by hypothermia. Animals were perfused briefly with 0.9% saline followed by a solution of cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) via the ascending aorta from the left ventricle for 10 minutes. The animals were fixed by immersion in the same fixative for 10 to 20 days at room temperature. Crystals of DiI picked up on tips of glass micropipettes were then placed into the rostral edge of the SC along the whole mediolateral extent of one lobe. Placements were made tangentially along this border to ensure that the maximum number of retinal axons entering the SC would be labelled. In older animals, the cortex was removed before DiI placement. A small piece of alfoil was inserted down the midline to stop spread of DiI to the other side of brain.

Histology

For DiI labelling. After a 3 to 14 months transport period, depending on age, the corneas, lenses and vitreous were dissected, followed by removal of the sclera, choroid and optic nerve. The retina with vitreal surface uppermost was then flat-mounted onto a gelatinized slide by making several radial splits in the periphery. The orientation of the retina was determined by the pigment line. The pigment line (Fig.3.1), which represents the demarcation line between light and heavy pigmentation, crosses the temporonasal pole (Flett, et al., '88). In general, this line became visible, with the appearance of heavy pigmentation in ventral

retina, from 28 days after birth. An angle between the pigment line and the nasal line (the line linking the nose and the centre of the eye) was used to predict the position of the pigment line and then to mark the orientation of retina. This angle, an average of 45^0, was obtained from measurements in twenty seven eyes, which were from animals aged 37 days or older. Antifade buffer, 2% n-propyl gallate and 80% glycerol in 0.1 M phosphate buffer, pH 7.4 (Nakamura and O'Leary, '89), was used as medium under the coverslip. The wholemounts of retina were then examined in a Leitz fluorescence microscope with an excitation filter of M2, Bp546/14. In some cases, the SC with the DiI placement was sectioned tranversely at 100 μm, on a vibratome onto 0.1 M phosphate buffer. The sections were observed to determine the position of DiI crystals.

For CFV staining. Sixteen fixed retinas were obtained from pouch young ranging in age from 5 to 45 days. A dorsal scalpel mark was made for orientation prior to eye removal. After the cornea and lenses were dissected from eyes, the eye balls embedded in a mixture of gelatin-albumin and glutaraldehyde, were sectioned on a freezing microtome at 40 μm (chapter 2). The sections through the retina were air-dried overnight. Subsequently, they were stained with a solution of 0.5% cresyl fast violet for 5 minutes, then dehydrated in graded alcohols, cleared in xylene and mounted in depex. Every second or third section, depending on age, was observed under a Leitz microscope.

Analysis

For DiI labelling. Mostly, the observations from the wholemounts of retina were made by using the NeuroTrace computer system (Massachusetts Institute of Technology, Cambridge, Massachusetts, USA),

which was combined with a Leitz fluorescence microscope (Fig.3.2). A drawing tube attached to the microscope superimposed a view of the computer monitor on the field of view of the microscope. By means of a cursor, outlines of the retina could be drawn first and the position of individual ganglion cells marked at a variety of magnifications using as many fields of view as required. Images were collected and stored and then printed out in a single drawing. Total numbers of the retinal ganglion cells recorded could be counted automatically and the density map of the labelled cells could be reconstructed immediately by the NeuroTrace program. This program provided a rapid, direct and detailed recording method for the present experiment.

In a few cases of older animals (=>28 days) because of the great number of ganglion cells, retinas were examined by a fluorescent microscope fitted with a calibrated eyepiece graticule which delineates squares of 0.5 x 0.5 mm^2 on the retina. Counts were made with a 25x objective on an area of 0.03 mm^2 from every square or at 0.5 mm intervals. The number of labelled ganglion cells in each square and the total numbers of labelled ganglion cells in the whole retina was calculated. Subsequently, an isodensity map was constructed. The area of retina covered by labelled ganglion cells was obtained by linking the most peripherally labelled ganglion cells by straight lines and measuring the enclosed area. This was expressed as a percentage of the total retinal area.

For CFV staining. The sections of retina were drawn by camera lucida and the position of the ganglion cell layer at the edge of the retina was marked. To demonstrate the global distribution of ganglion cells, the sections showing the ganglion cell layer were then restacked to reconstruct the whole retina. Lines were drawn joining the extent of the retina and of the ganglion cell layer, respectively. The map displaying the extent of the ganglion cell layer in the retinal area was obtained at different stages of development.

RESULTS

The distribution of ganglion cells projecting to the SC contralaterally and ipsilaterally and the extent of the ganglion cell layer in developing retina is described in detail.

In the present study, DiI placements in the SC were made in thirty one animals (see Materials and Methods). Twenty cases, with convincing efficiency of labelling, of both contralateral and ipsilateral retinas, are described at different ages ranging from 4-5 (n=3), 9 (n=2), 11-15 (n=5), 16 (n=3), 28-32 (n=4) and 41-46 (n=3) days. All cases are included in Table 3.1. Those considered less successful are indicated in the table and are also discussed at the appropriate age group. Good labelling efficiency could be achieved by increasing the transport period. The transport period was prolonged with age in order to obtain the maximum labelling.

After examining the DiI deposits in the SC, no DiI crystals were found ectopically in each case. In the cases where the transverse sections of the SC with DiI placements were cut ectopic DiI crystals were not detected, and the fluorescence in the SC and the retrogradely labelled axons in the optic tract were much brighter than in those areas bordering the deposit site.

The table and all the figures for this chapter are grouped together at the end of the results section.

4-5 days

Distribution of ganglion cells projecting to the SC. In two cases, ganglion cells were retrogradely labelled only in the retina contralateral to the dye placement site in the SC. Ganglion cells were sparse and had few dendrites (Fig.3.3). They were situated centrally in the dorsal and slightly temporal retina, extending to roughly one-half of the radius of the retina. No ganglion cells were found ipsilaterally (Fig.3.4). In the third case, presumably representing a slightly more advanced stage, more cells projecting contralaterally were detected centrally, mainly in the dorsal and temporal and cells were first seen in the nasal retina. Ganglion cells ipsilateral to the DiI deposit in the SC were first labelled. They were also seen mainly in the dorsal retina (Fig.3.5). The contralaterally projecting ganglion cells covered 29% of the retinal area. On average, seventy nine ganglion cells were labelled in the contralateral and three in the ipsilateral retina, respectively, at this stage.

Extent of ganglion cell layer. A complementary observation on the extent of the ganglion cell layer was obtained with CFV staining at 5 days. The inner plexiform layer is distinct across the retina except towards the ora serrata. The ganglion cell layer could be distinguished by virtue of the determination of position, size, shape and staining intensity of cell bodies (Fig.3.6). The extent of the ganglion cell layer reconstructed in a map showed that this layer was present mainly over the dorsal half of retina, with a percentage occupation of 51% in the retina. The ganglion cell layer extended further peripherally in the dorsal retina than in the rest of the retina (Fig.3.7). This shape of the extent of the ganglion cell layer roughly related to the distribution of the contralateral ganglion cells retrogradely labelled by DiI although the ganglion cells projecting to the SC did not

extend as far peripherally as the ganglion cell layer at this early stage (Fig.3.8).

8-9 days

Distribution of ganglion cells projecting to the SC. In the two successful cases, contralaterally labelled ganglion cells increased rapidly in the nasal as well as in the dorsal and temporal retina. Particularly, more cells began to concentrate in the temporal retina. Sparse numbers of labelled ganglion cells were first seen in the ventral half of the retina. Cells in the periphery of the contralateral retina remained unlabelled. The unlabelled periphery was larger ventrally (Fig.3.9). The average of total labelled cells rose to 512. The region covered by retrogradely labelled ganglion cells increased to 77% of retinal area. On the ipsilateral side, more labelled cells (19, on average) were seen scattered in each quadrant of the retina with a preference for the dorsal half of the retina. They were still distributed more or less centrally, surrounding the optic disc (Fig.3.9). In the three unsuccessful cases, very few cells were labelled.

Extent of ganglion cell layer. By using CFV staining methods, 72% of the retinal area was found to be covered by ganglion cells, corresponding to the similar region covered by contralaterally labelled cells with DiI (Fig 3.7, 3.8).

11-15 days

Distribution of ganglion cells projecting to the SC. In the five successful cases, the area covered by contralaterally labelled ganglion cells increased in each of the four quadrants of the retina. More cells were

spread temporally and dorsally and reached close to the periphery. The number of the cells in the nasoventral retina was much less than in other regions and labelled cells were sparse or absent in the periphery (Fig.3.10). Labelled cells occupied contralaterally 85% of retinal area. The mean of ganglion cells projecting contralaterally at this age group reached to 878. The distribution of ganglion cells ipsilateral to the SC was found to be qualitatively similar to that at younger stages, with sparse labelled cells distributed mainly over the dorsal half of the retina (Fig.3.10). On average, only 29 ganglion cells were counted ipsilaterally. In one unsuccessful case, very few cells were labelled contralaterally and none ipsilaterally.

Extent of ganglion cell layer. From the reconstructed map of the ganglion cell layer stained with CFV, ganglion cells were found to occupy 83% of the retinal area on average, similar to the coverage of contralaterally labelled cells with DiI (Fig.3.7, 3.8).

16-18 days

Distribution of ganglion cells projecting to the SC. By 16 days, a dramatic change in the distribution pattern of ganglion cells was revealed in two of the successful cases. The first signs of a visual streak beginning to form were seen, in which DiI-labelled ganglion cells projecting to the contralateral SC were spread predominantly in the dorsal and temporal retina along a band orientated temporonasally, just above the optic disc. The adult has a well-defined visual streak oriented across the temporonasal pole of the eye, containing an area centralis with highest ganglion cell density in the temporal retina (Tancred, '81; Wong et al., '86). Sparse cells were seen to extend more peripherally. These were still particularly sparse in nasoventral retina (Fig.3.11). It was seen clearly that

distinct fibre fascicles run from the optic disc towards the ganglion cells scattered around the retina and more axons were seen dorsotemporally (Fig.3.12). The dendrites of ganglion cells were labelled clearly (Fig.3.13). Ipsilaterally, an adultlike pattern began to appear, in which the majority of labelled ganglion cells were already concentrated in a crescent in the periphery of the temporoventral retina and cells were absent or sparse elsewhere (Fig.3.11), with axons running from the optic disc to the labelled cells (Fig.3.14). The third successful case represents an intermediate stage beween the previous age groups and the other two cases. The visual streak was not yet significant contralaterally, although more labelled ganglion cells were distributed dorsally and temporally. In addition, like the other two cases, there was a concentration of cells in temporoventral retina ipsilaterally, with a few ganglion cells scattered outside the region (Fig.3.15). Thus, the findings suggested that the mature pattern of retinal ganglion cell distribution begins to appear as early as 16 days in the developing wallaby. At this age, the average number of contralaterally labelled cells reached 976, with coverage of 86% of retinal area. As many as 54 cells on average were counted ipsilaterally. In two of the unsuccessful cases, there were few labelled cells contralaterally and these were mainly in the dorsotemporal retina. Ipsilaterally, cells were few and were in the dorsal or ventral retina. In the third unsuccessful case, although there were large numbers of labelled cells, contralaterally they were only in the dorsal and central retina. Ipsilaterally, cells were mainly in the temporoventral crescent as described for two of the successful cases, with a few cells scattered outside this area.

Extent of ganglion cell layer. Ganglion cells stained by CFV spread to occupy 86% of retinal area at 17-18 days, the same as the region covered by contralaterally labelled cells with DiI at the similar age (Fig.3.7, 3.8).

26-36 days

Distribution of ganglion cells projecting to the SC. At 28-32 days, in the four successful cases, there was a great increase in the number of ganglion cells projecting contralaterally to the SC. The ganglion cells were distributed mainly in the temporal and dorsal retina, with a lower density extending into the nasal retina, to form a putative visual streak. The density of labelled cells was highest in the central temporodorsal retina and gradually fell off towards the nasoventral retina and towards the periphery of each quadrant of the retina (Fig.3.16). Many more labelled ganglion cells with clearly defined dendrites were found than those at younger stages (Fig.3.17). Contralaterally, the number of labelled cells reached 15078 on average and the area occupied by the labelled cells reached to 95% of retina. The distribution of retrogradely labelled ganglion cells seen in the retina ipsilateral to the SC showed a qualitatively similar pattern to that seen at the previous age. In three of the cases, labelled cells were restricted totally to the distinct crescent-shaped regions in the periphery of the temporoventral retina (Fig.3.18). Only in one case, were a few cells also seen to be scattered centrally around the optic disc (Fig.3.16). Quantitatively, the number of labelled cells increased greatly to 2345 by this stage. In the two unsuccessful cases, although many cells were labelled, contralaterally they were only seen in central retina. Ipsilaterally, labelled cells were totally confined to the temporoventral crescent as those distributed in the successful cases.

Extent of ganglion cell layer. Results, obtained from reconstruction of sectioned retinas stained by CFV, demonstrated data compatible with DiI labelling contralaterally. Beween 26 to 32 days, this coverage remained

unchanged. The overall extent of the ganglion cell layer covered 95% of the retina (Fig.3.7, 3.8).

41-46 days

Distribution of ganglion cells projecting to the SC. There was no dramatic change in the distribution pattern of ganglion cells projecting contralaterally in the three successful cases. The majority of ganglion cells contralateral to the SC were concentrated centrally on each of four quadrants, mainly in the temporal and dorsal retina to form a temporonasally aligned visual streak. The rest of the cells were spread sparsely in the periphery of each quadrant. A distinct crescent with the axons arcing towards the optic disc was seen ipsilaterally. At this stage, no labelled cells were seen scattered outside this crescent in all the cases examined (Fig.3.19). However, cell counts revealed a dramatic fall in the number of labelled cells both contralaterally and ipsilaterally. The number of labelled ganglion cells contralateral and ipsilateral to the SC dropped to 7571 and 409, respectively, although the labelled region containing ganglion cells contralateral to the SC still covered 95% of retina. During the postnatal development, there was a 50% reduction in the number of crossed cells and an 83% reduction in uncrossed cells, in comparison with the peak at 28-32 days. In one unsuccessful case, there was no labelling contralaterally and only a few cells ipsilaterally. In the other case, although reasonable numbers of cells were labelled contralaterally, they were only seen in the central retina. Ipsilaterally, cells were confined to the temporoventral crescent as in the successful cases.

Extent of ganglion cell layer. The extent of ganglion cells stained by CFV remained similar to that seen at the previous stage, occupying 96% of

retinal area at 45 days. Comparably, this extent was similar to the region covered by contralaterally labelled cells with DiI at the similar age (Fig.3.7, 3.8).

Table 3.1 Transport period of DiI and numbers of ganglion cells with crossed and uncrossed axons in the retina from 4 to 46 days

Ages (days)	Transport period (months)	GC with crossed axon	GC with uncrossed axon
4	3	35	0
4	4	153	7
5	4	50	2
Mean		79	3
9	2	535	9
9	6	489	29
Mean		512	19
8*	3	30	0
8*	3	76	4
8*	5	0	0
11	3	860	11
12	5.5	618	61
12	3	777	22
13	3	1140	38
13	4	873	12
Mean		878	29
12*	3	294	0
16	5	1238	89
16	5	993	48
16	5	528	24
Mean		976	54
16*	3	127	16
16*	3	461	2
16*	4	1145	161
28	6	16467	1960
28	8	10000	1661
32	6	13846	2150
32	9	20000	3610
Mean		15078	2345
28*	3.5	many (central retina)	many (T-V crescent)
36*	6	many (central retina)	many (T-V crescent)
41	14	4673	471
44	14	9039	326
46	14	9000	430
Mean		7571	409
45*	7	0	4
41*	8	many (central retina)	many (T-V crescent)

GC: ganglion cell. T-V: temporal-ventral.

* indicates unconvincing cases

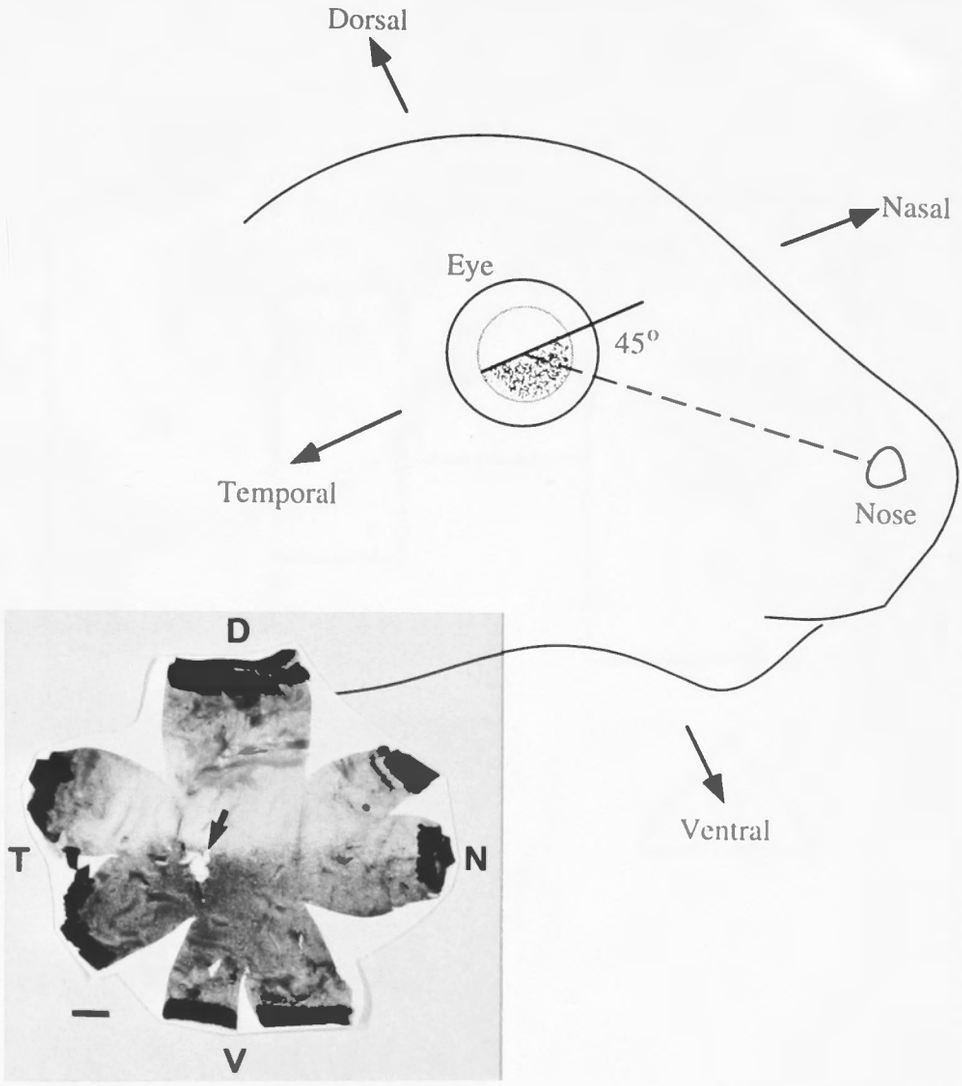


Figure 3.1 Alignment of pigment line

A line drawing of an animal's head is shown. The pigment line in the retina, which indicates the border between light and dark pigmentation across the temporonasal pole of the eye, is at an angle with an average of 45° to a line linking the nose and the centre of the eye. This bright-field view shown in the inset demonstrates that the pigment line between light (upper) and heavy (lower) pigmentation crosses the temporonasal pole, just superior to the optic disc indicated by an arrow. D: dorsal; T: temporal; N: nasal; V: ventral. Bar: 1 mm.

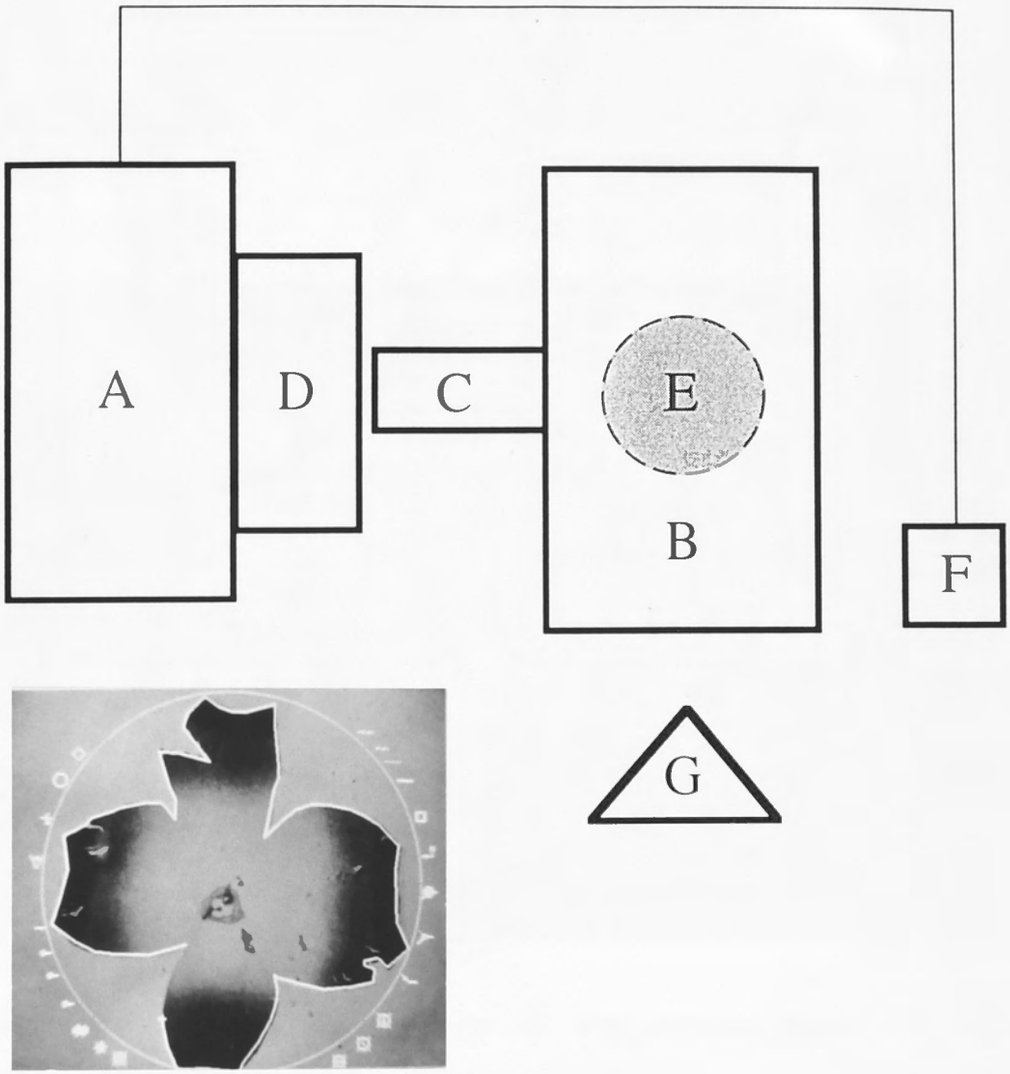


Figure 3.2 Plan of the recording system

NeuroTrace computer system (A) is combined with a Leitz fluorescence microscope (B) by a drawing tube (C) attached to the microscope. A view of the computer monitor (D) is superimposed on a field of view of the microscope (E), which is shown in the inset. A retinal wholemount is outlined in the drawing monitor. By moving a cursor (F), the user (G) can draw features of interest.

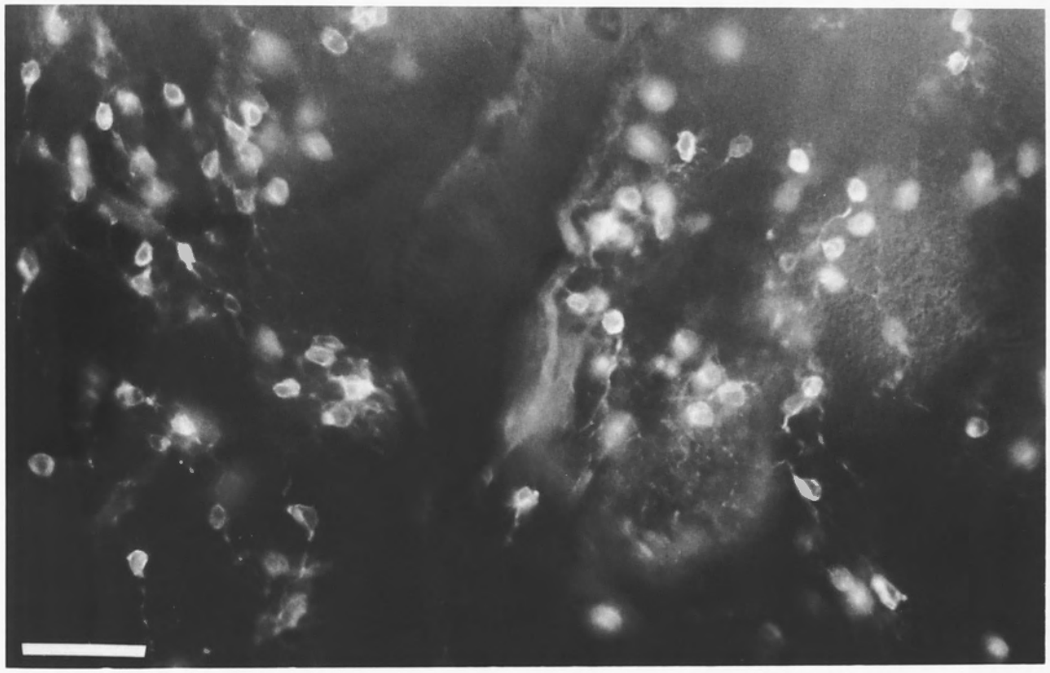


Figure 3.3 Fluorescent image of the retinal ganglion cells projecting contralaterally to the SC at 4 days

Individual ganglion cells with dendrites are clearly defined in the dorsal retina by using DiI as a retrograde tracer in the contralateral SC. Bar: 50 μm.

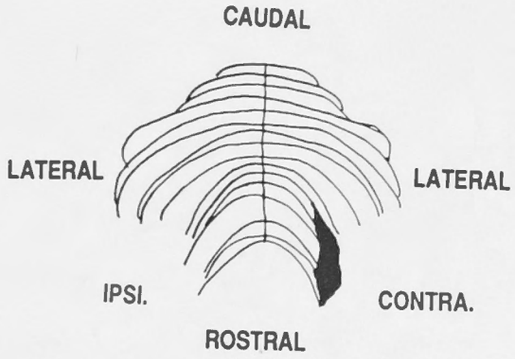
Fig 3.4 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the retinal innervation to the SC at 4 days

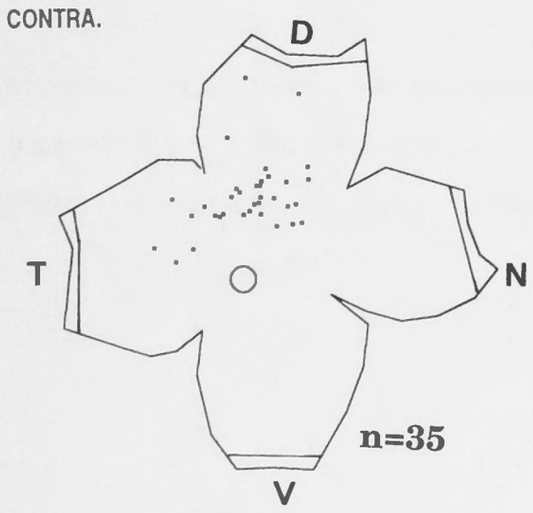
Bottom left: Position of ganglion cells in the retina contralateral to the SC with DiI placement. Outline of the wholemount of retina with labelled ganglion cells shown by small spots. Open circle marks the optic disc (OD). Contralaterally labelled ganglion cells are distributed centrally in the dorsal and slightly temporal retina.

Bottom right: Position of ganglion cells in the retina ipsilateral to the SC with DiI placement. No ganglion cells are detected ipsilaterally.

Top: Retinal projection to the SC. In this and similar subsequent figures, the distribution of labelled axons in the SC after an eye injection of HRP at the same age is shown for comparison. These results are fully described in the previous chapter. The black area indicates the projection to the contralateral SC. Retinal axons enter the contralateral SC rostrocaudally. No axons are seen to enter the ipsilateral SC. D: dorsal; T: temporal; N: nasal; V: ventral; Contra: contralateral; Ipsi: ipsilateral; n: numbers of ganglion cells retrogradely labelled by DiI. Bars: 1 mm.

4 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL


CONTRA.
D
T
N
V
n=35

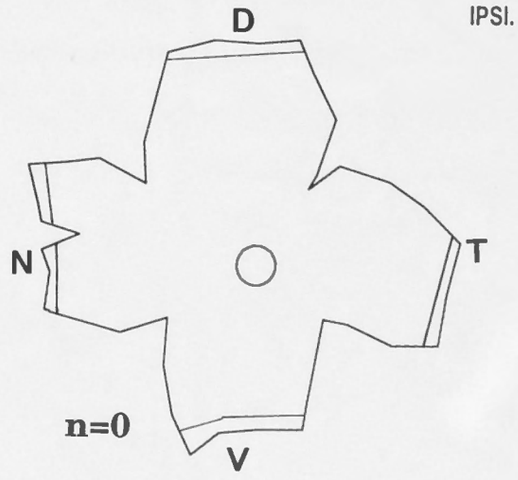
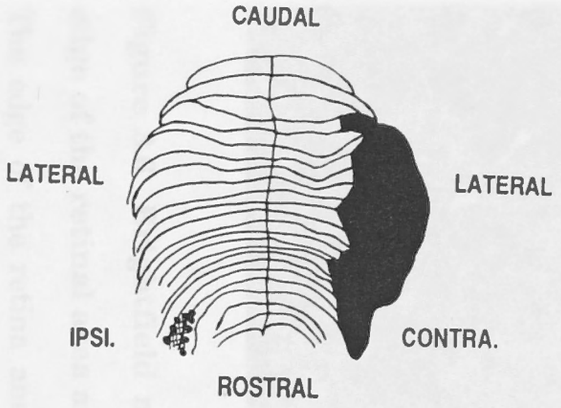
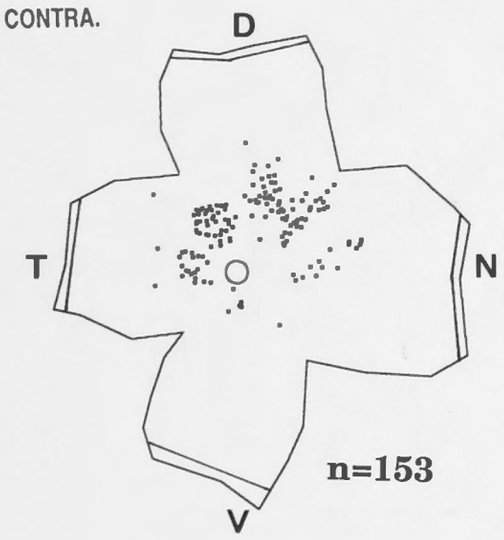

D
IPSI.
N
T
n=0
V

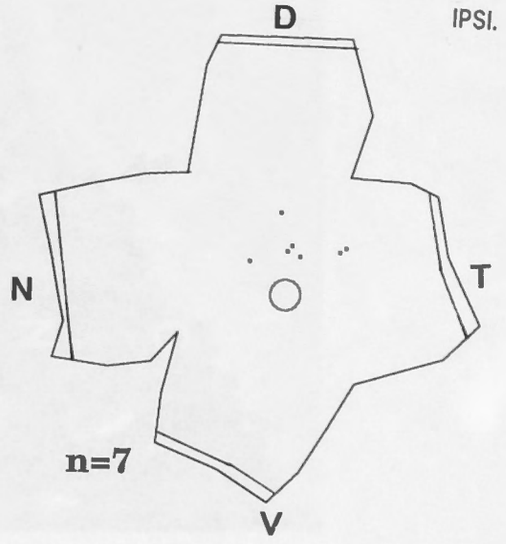
Figure 3.5 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the retinal innervation to the SC at 4-5 days

Conventions are the same as for figure 3.4. In addition, the cross hatching indicates the area of the projection to the ipsilateral SC. More ganglion cells in the retina contralateral to the SC are spread more centrally around the optic disc, mainly in the dorsal and temporal retina. A few ganglion cells are first labelled ipsilaterally. They are distributed centrally and dorsally. Retinal axons occupy the lateral half of the SC contralaterally, and extend over most of the rostrocaudal extent except for the far caudal pole. Ipsilaterally, axons begin to invade the rostrolateral pole of the SC. Bars: 1 mm.

4-5 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL


CONTRA.
D
T
N
V
n=153


D
IPSI.
N
T
n=7
V

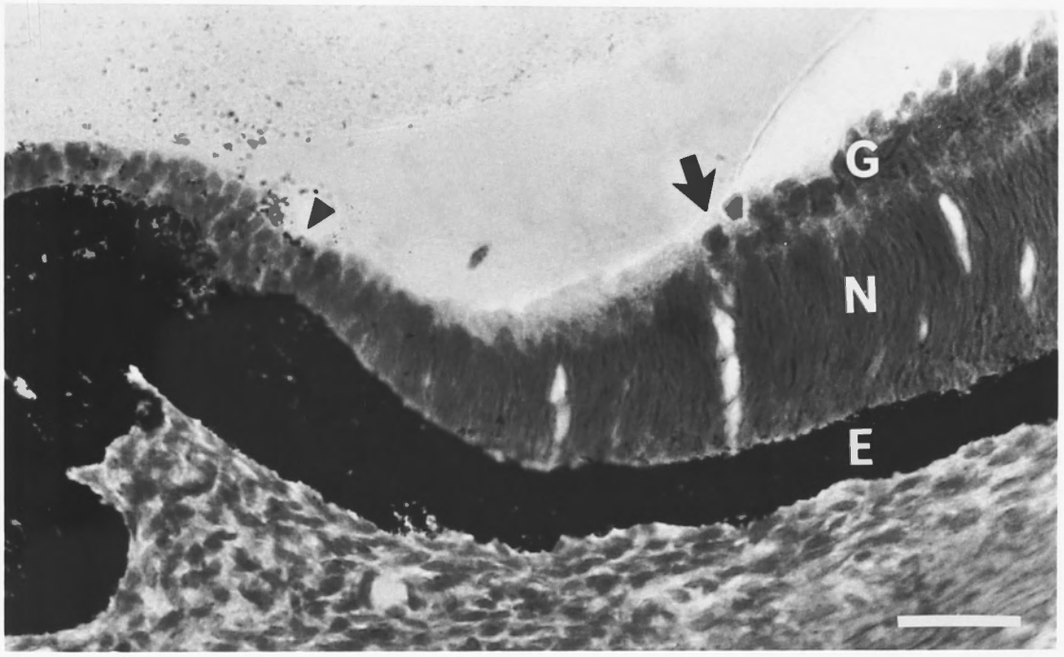
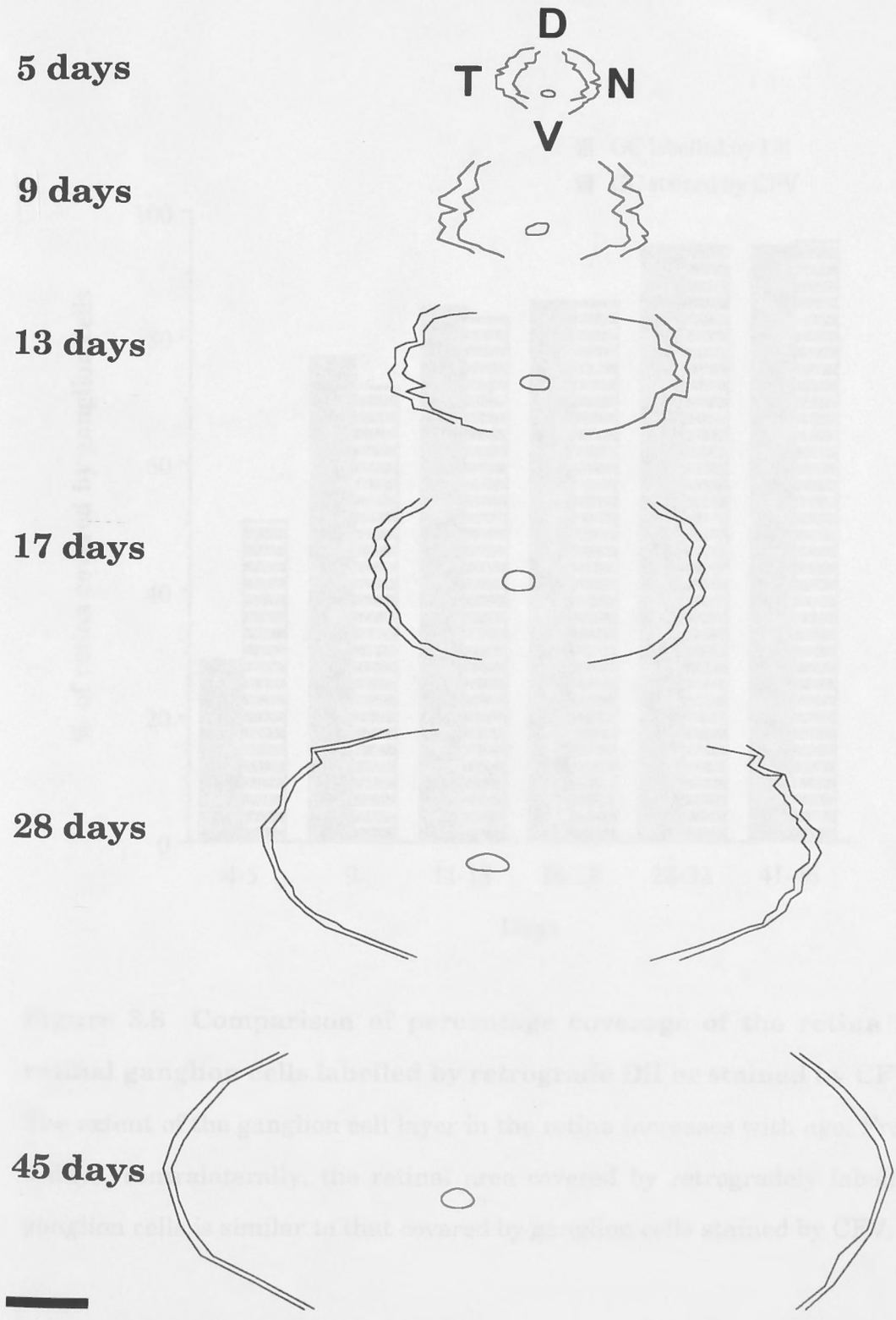


Figure 3.6 Brightfield micrograph of sectioned eye showing the edge of the retinal area and ganglion cell layer at 5 days

The edge of the retina and the edge of the ganglion cell layer (G) are marked by an arrow head and an arrow, respectively. N: neuroblast layer; E: pigment epithelium. Bar: 50 μm.

Figure 3.7 Reconstructed map of sectioned retinas showing the extent of the ganglion cell layer from 5 to 45 days

The sections of retina showing the position of the ganglion cell layer are restacked to reconstruct the whole retina. Open circle marks the optic disc. The inner line indicates the distribution of the ganglion cell layer and the outer line indicates the outline of the retina. Initially, the ganglion cells are distributed across the central region of temporal and nasal retina and extend close to the dorsal periphery. Gradually, ganglion cells cover more of the retina with age. D: dorsal; T: temporal; N: nasal; V: ventral. Bar: 1 mm.


5 days
D
T
N
V
9 days
13 days
17 days
28 days
45 days

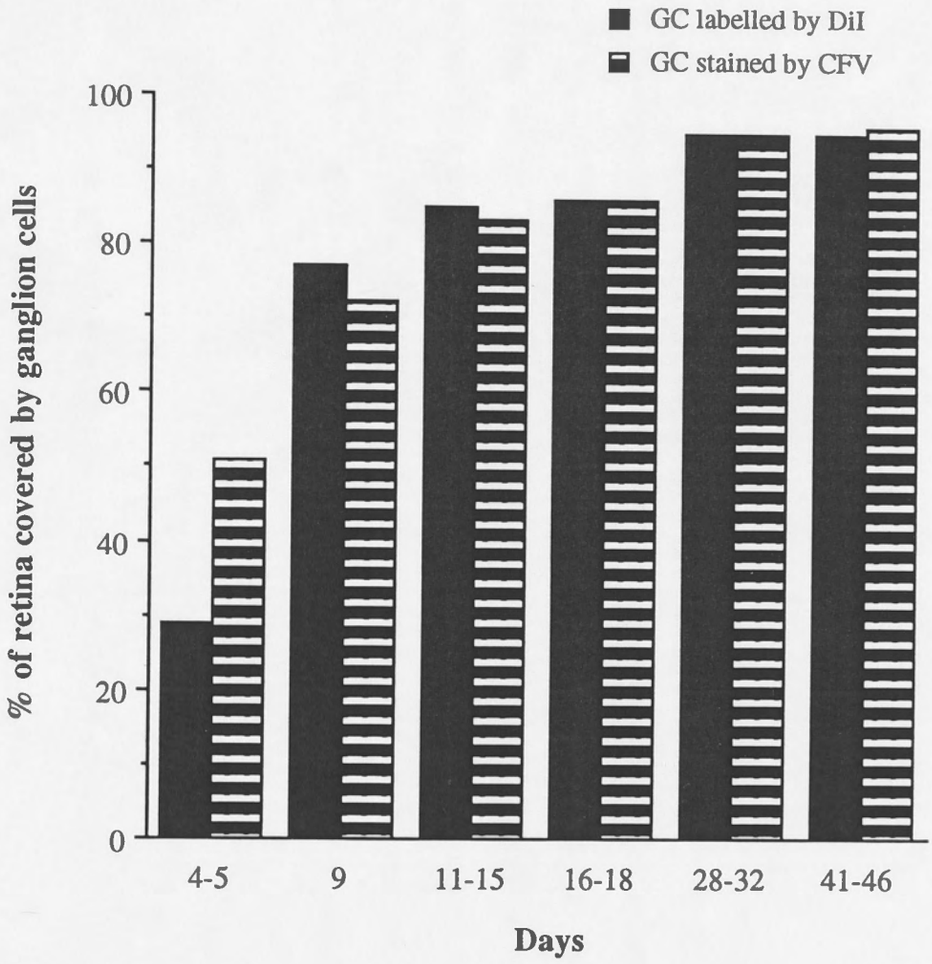


Figure 3.8 Comparison of percentage coverage of the retina by retinal ganglion cells labelled by retrograde DiI or stained by CFV

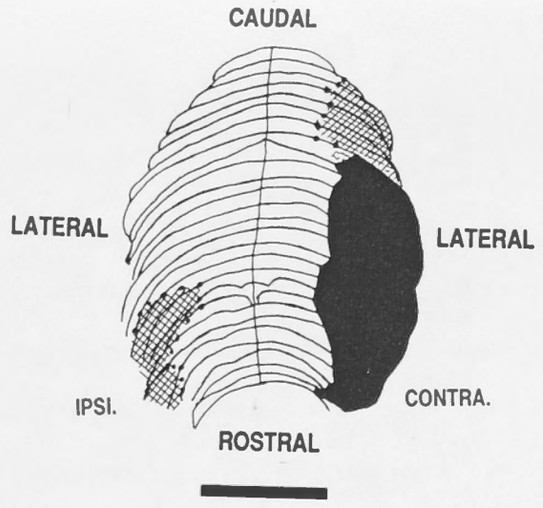
The extent of the ganglion cell layer in the retina increases with age. From 9 days, contralaterally, the retinal area covered by retrogradely labelled ganglion cells is similar to that covered by ganglion cells stained by CFV.

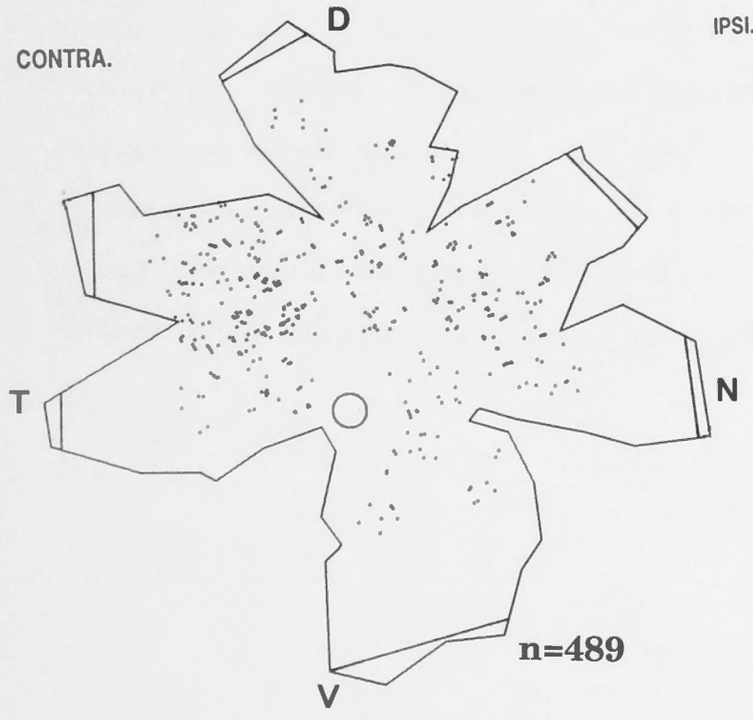
Figure 3.9 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the retinal innervation to the SC at 8-9 days

Conventions are the same as for figure 3.4 and 3.5. Many ganglion cells contralateral to the SC are labelled in the temporal, dorsal and nasal retina. Labelled cells are sparse ventrally. The extreme periphery of the retina remains unlabelled. More ganglion cells than at earlier ages, ipsilateral to the SC, are scattered diffusely in the more central area of each quadrant of the retina. Retinal axons in the contralateral SC extend more medially than at younger stages. The axons spread further caudally and become sparser at the caudal pole (indicated by cross hatching). Ipsilaterally, retinal axons are distributed rostrolaterally. Bars: 1 mm.

CAUDAL

8-9 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL


CONTRA.
D
T
N
V
n=489

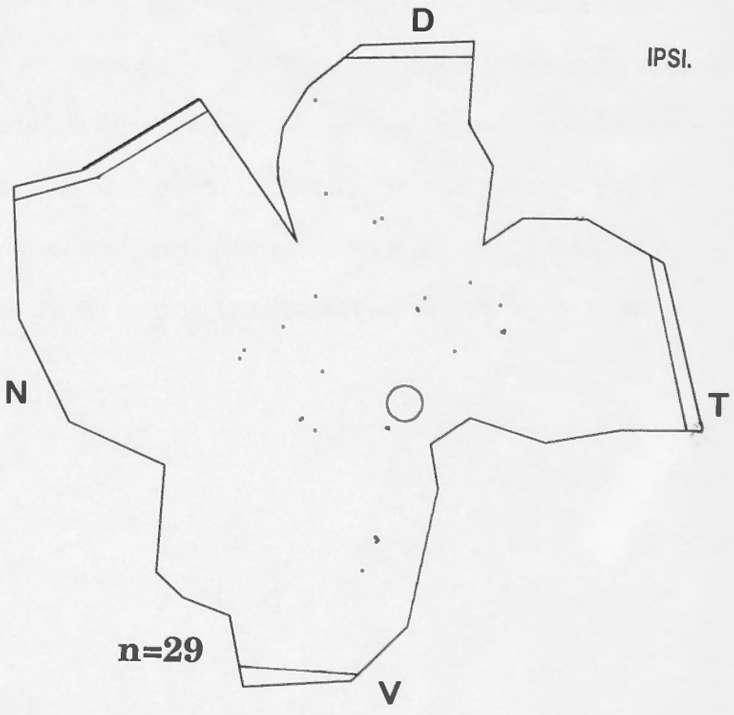
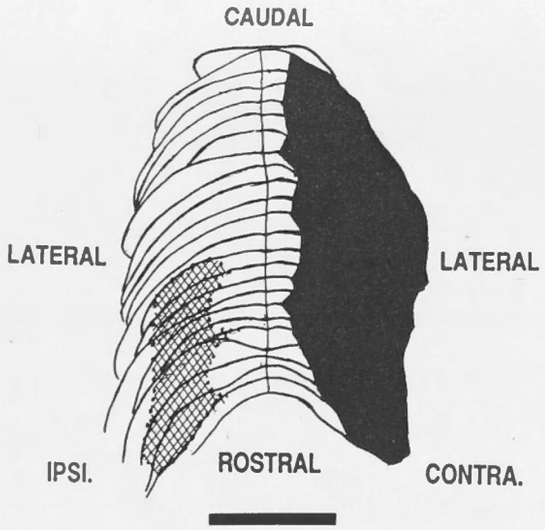

D
IPSI.
N
T
n=29
V

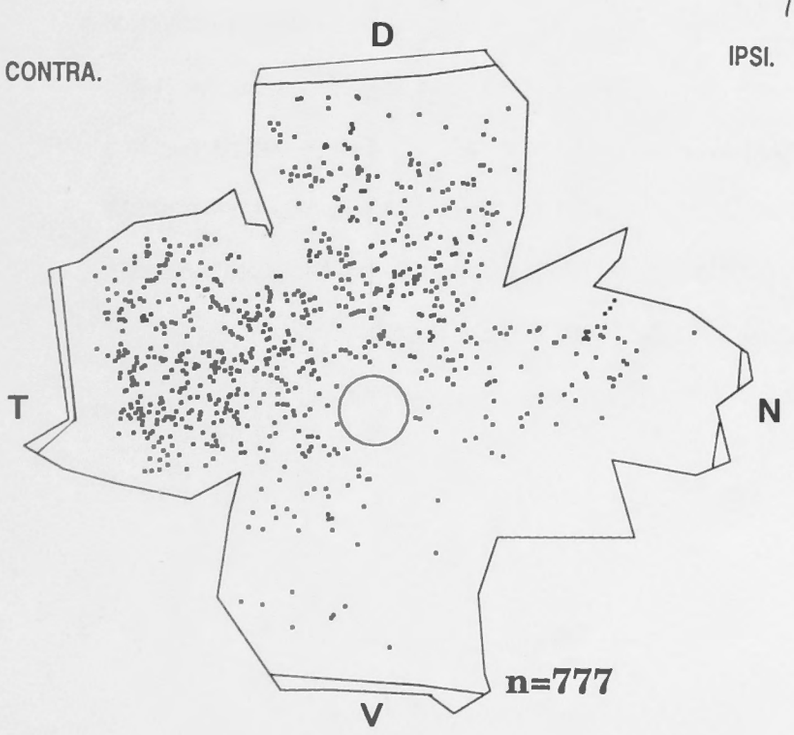
Figure 3.10 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the retinal innervation to the SC at 12 days

Conventions are the same as for figure 3.4 and 3.5. Many ganglion cells are spread contralaterally over most of the retina with the exception of the far periphery. In the main, they are distributed dorsally and temporally. The number of labelled cells in the nasoventral retina is less than that in other regions. The distribution of ganglion cells ipsilateral to the SC is similar to that seen at earlier stages. Labelled cells are sparse over the retina and mainly in the dorsal retina. The retinal projection to the contralateral SC covers most of the rostrocaudal extent and four fifths of the lateromedial extent of the SC. Ipsilaterally, the retinal projection is distributed rostrolaterally. Bars: 1 mm.

CAUDAL

12 days


CAUDAL
LATERAL
LATERAL
IPSI.
ROSTRAL
CONTRA.


CONTRA.
D
T
N
V
n=777

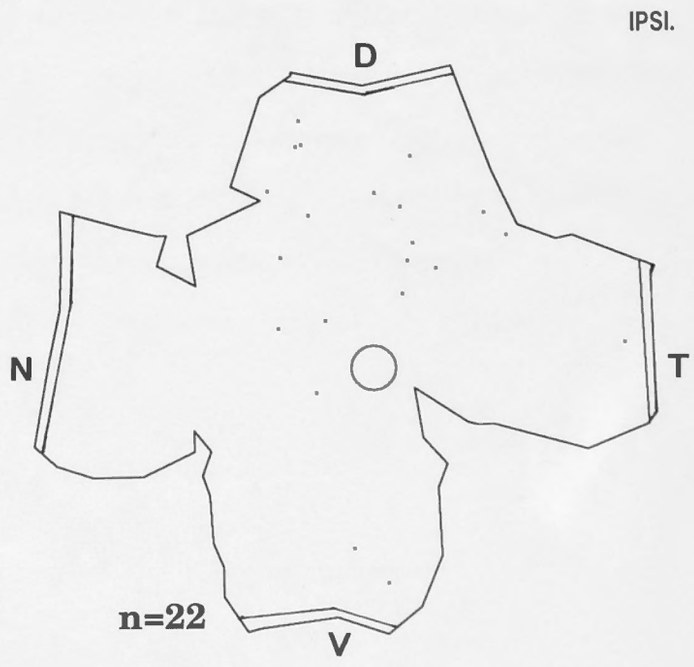
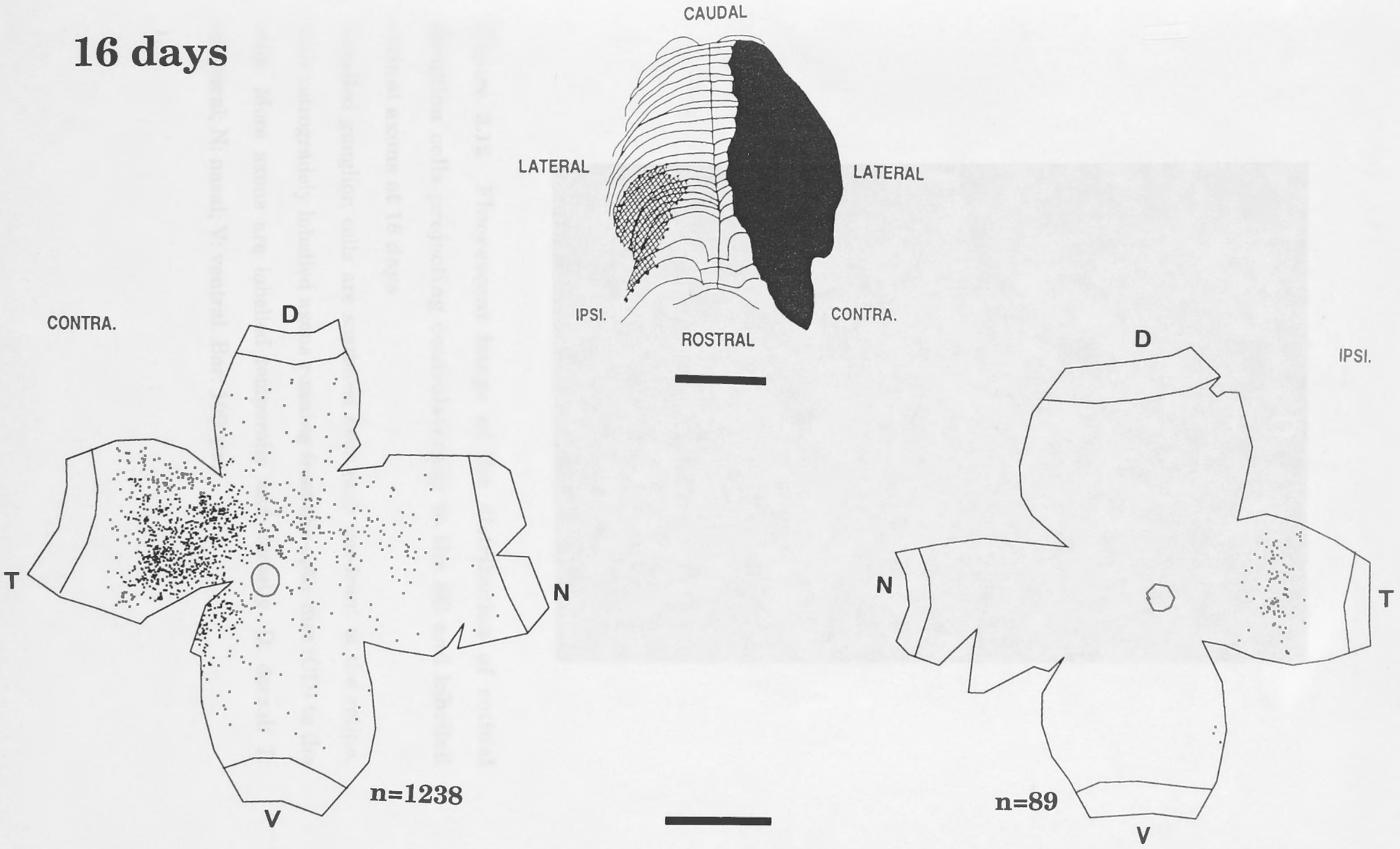

IPSI.
D
N
T
V
n=22

Figure 3.11 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the innervation to the SC at 16 days

Conventions are the same as for figure 3.4 and 3.5. This is an example of one of the two types of pattern seen at this age, representing a more advanced stage. The other example is shown in figure 3.15. Labelled ganglion cells in the retina contralateral to the SC are spread over most of the retina. The first sign of a visual streak is seen temporonasally in a band superior to the optic disc, containing an area centralis with the highest density of ganglion cells in the central temporodorsal retina. The number of cells falls off nasoventrally as well as peripherally. For the first time, a distinct crescent-shaped region formed by labelled ganglion cells is seen in the more peripheral part of temporoventral retina. In the contralateral SC, the retinal projection covers a slightly more extensive area than that seen at the previous age. Ipsilaterally, the retinal projection is still distributed rostrolaterally. Bars: 1 mm.

16 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL
CONTRA.
D
T
N
V
n=1238
D
IPSI.
N
T
V
n=89

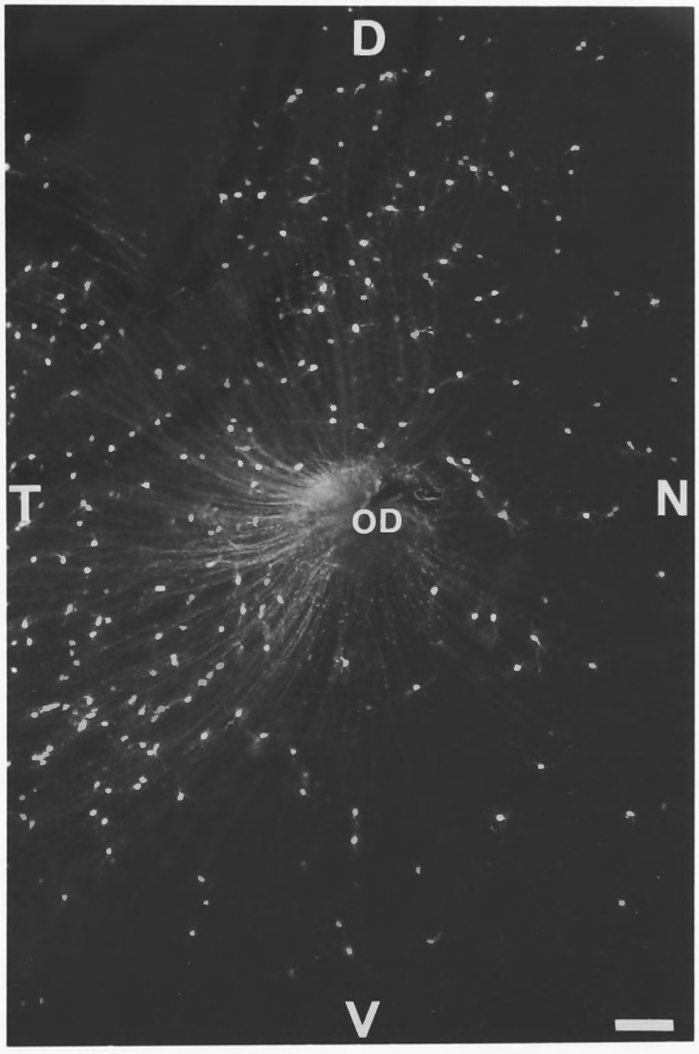


Figure 3.12 Fluorescent image of the distribution of retinal ganglion cells projecting contralaterally to the SC and labelled retinal axons at 16 days

Labelled ganglion cells are scattered over each quadrant of the retina, with retrogradely labelled axons running from the optic disc (OD) to the cells. More axons are labelled temporally and dorsally. D: dorsal; T: temporal; N: nasal; V: ventral. Bar: 100 μm.

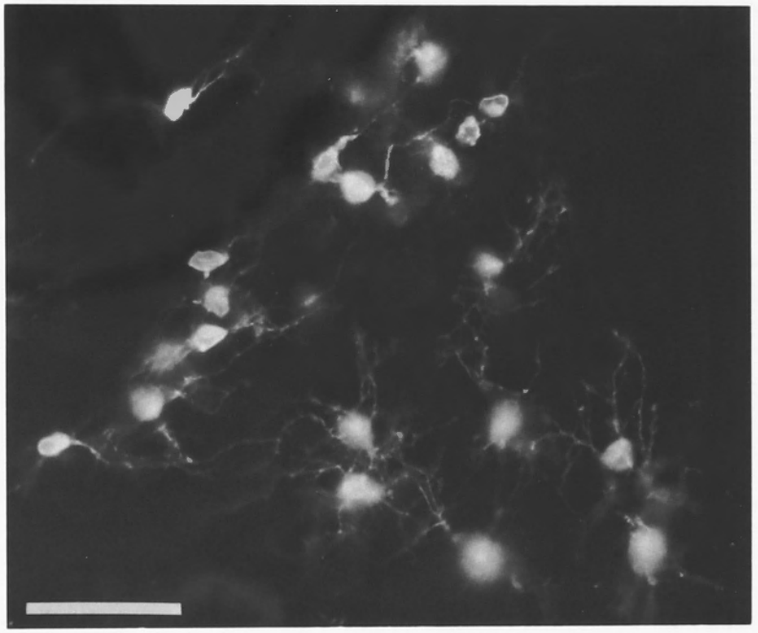


Figure 3.13 Fluorescent image of the retinal ganglion cells projecting contralaterally to the SC at 16 days

Individual ganglion cells with dendrites are clearly defined in the retina contralateral to the SC. Bar: 50 μm.

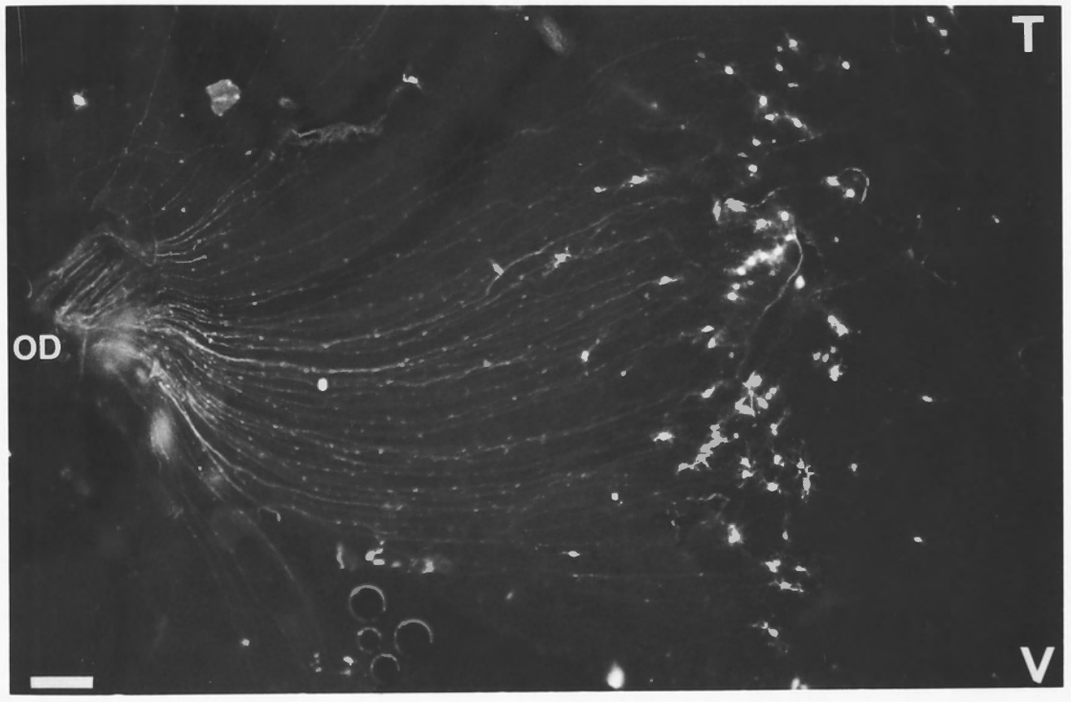


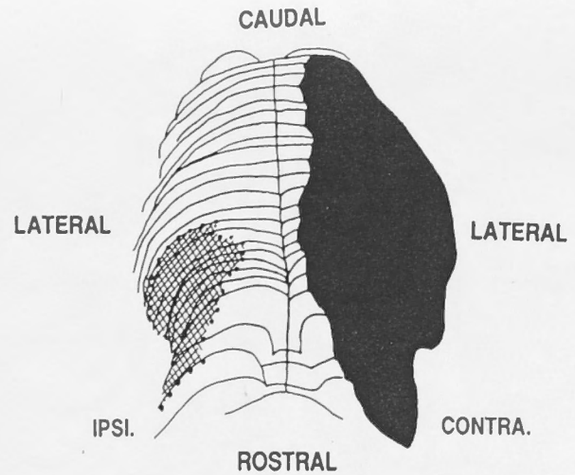
Figure 3.14 Fluorescent image of the distribution of retinal ganglion cells projecting ipsilaterally and labelled retinal axons at 16 days

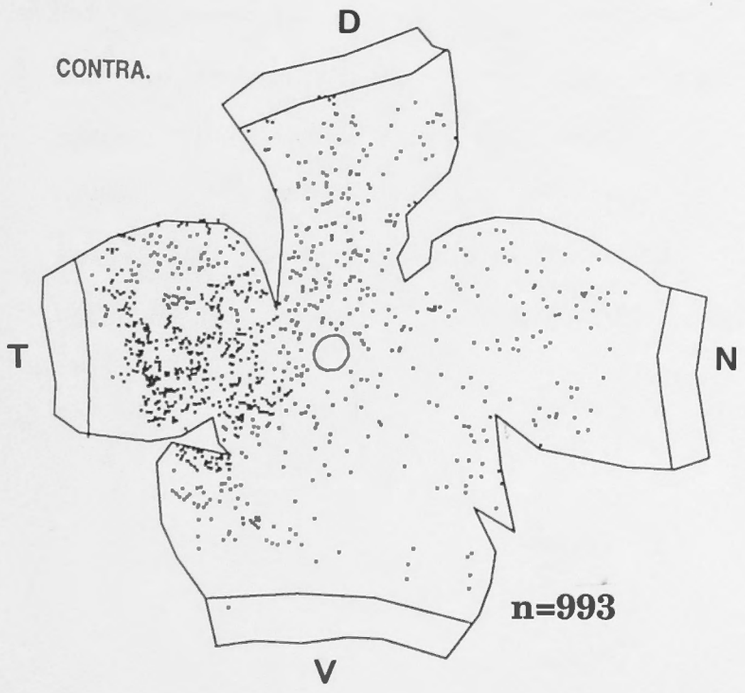
Labelled ganglion cells form a crescent-shaped region in the periphery of the temporoventral retina, with the labelled axons arcing towards the optic disc (OD). T: temporal; V: ventral. Bar: 100 μm.

Figure 3.15 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the innervation to the SC at 16 days

Conventions are the same as for figure 3.4 and 3.5. Many labelled ganglion cells are scattered contralaterally over most of the retina, with more distributed dorsotemporally. In this case a visual streak is not seen. Ipsilaterally, labelled ganglion cells are distributed in the temporal hemiretina with a preference for the periphery of the temporoventral retina, but the adultlike pattern seen in the other case at this age (Fig.3.11) is not obvious. The pattern of retinal projections to the SC is the same as in Fig.3.11. Bars: 1 mm.

16 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL


D
CONTRA.
T
N
V
n=993

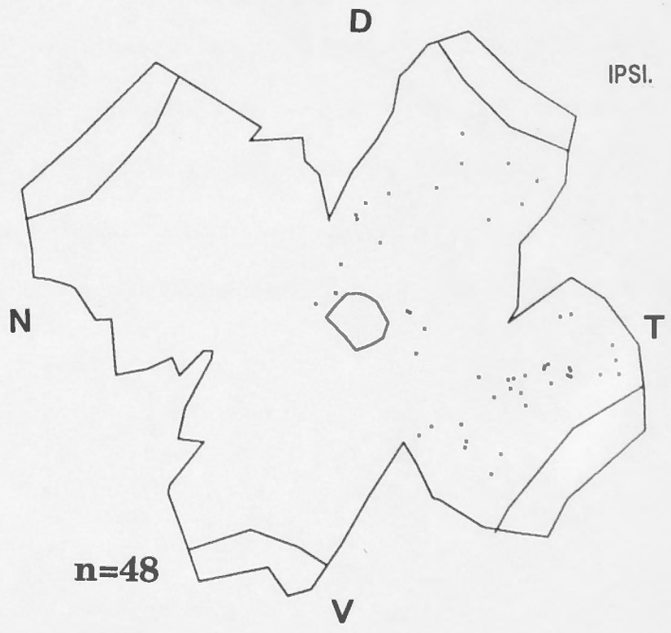
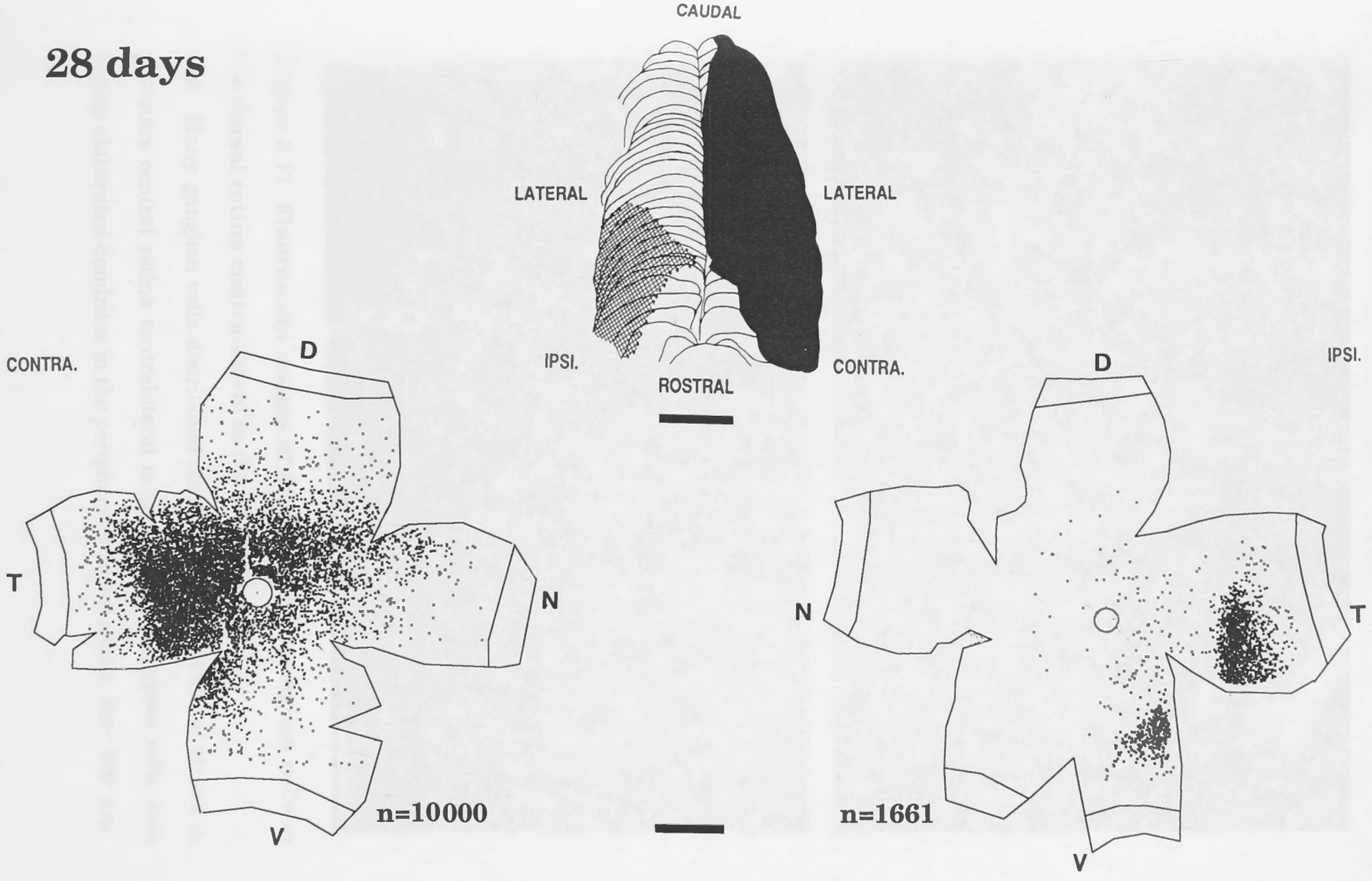

D
IPSI.
N
T
n=48
V

Figure 3.16 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the innervation to the SC at 28 days

Conventions are the same as for figure 3.4 and 3.5. A large number of ganglion cells are spread contralaterally over the whole retina, with an increasing number in the ventral retina. The density of labelled cells is highest in the central temporodorsal retina and gradually falls off nasoventrally. The visual streak and area centralis is more obvious than at the previous age. The cells in the periphery of the retina are sparse. The majority of labelled cells are confined ipsilaterally to a distinct crescent in the periphery of temporoventral retina. A few cells are scattered centrally, primarily in the temporal half. From this stage, the retinal projection extends to the medial border of the contralateral SC except for the far caudal pole. Fibres are spread more extensively in the rostrocaudal part of the ipsilateral SC. Bars: 1 mm.

28 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL
CONTRA.
D
T
N
V
n=10000
D
IPSI.
N
T
V
n=1661

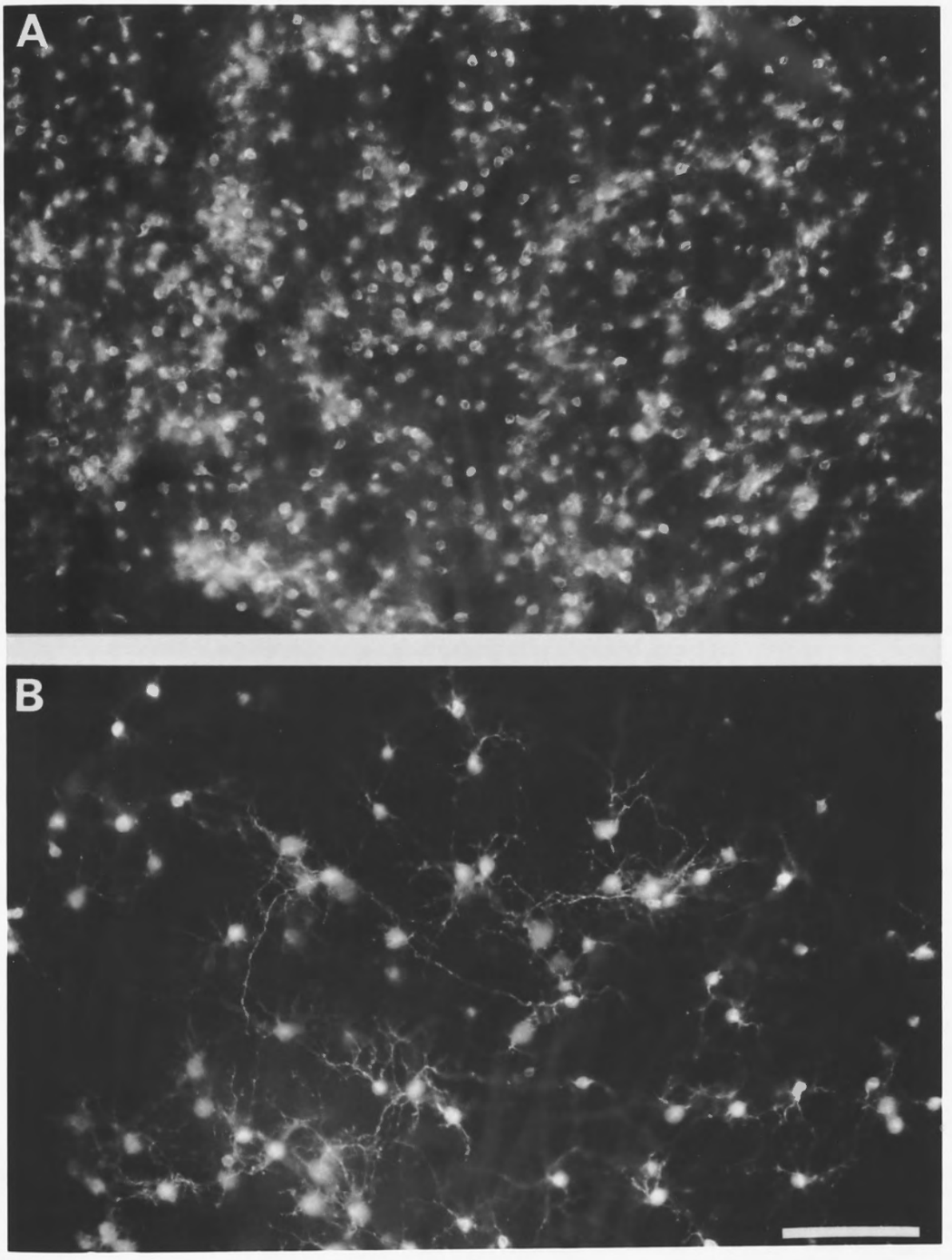


Figure 3.17 Fluorescent images of distribution of ganglion cells in the dorsal retina contralateral to the SC at 28 days

(A): Many ganglion cells distributed dorsally within the visual streak in the more central retina contralateral to the SC. **(B):** Ganglion cells with highly elaborated dendrites in the periphery of dorsal retina. Bar: 100 μm.

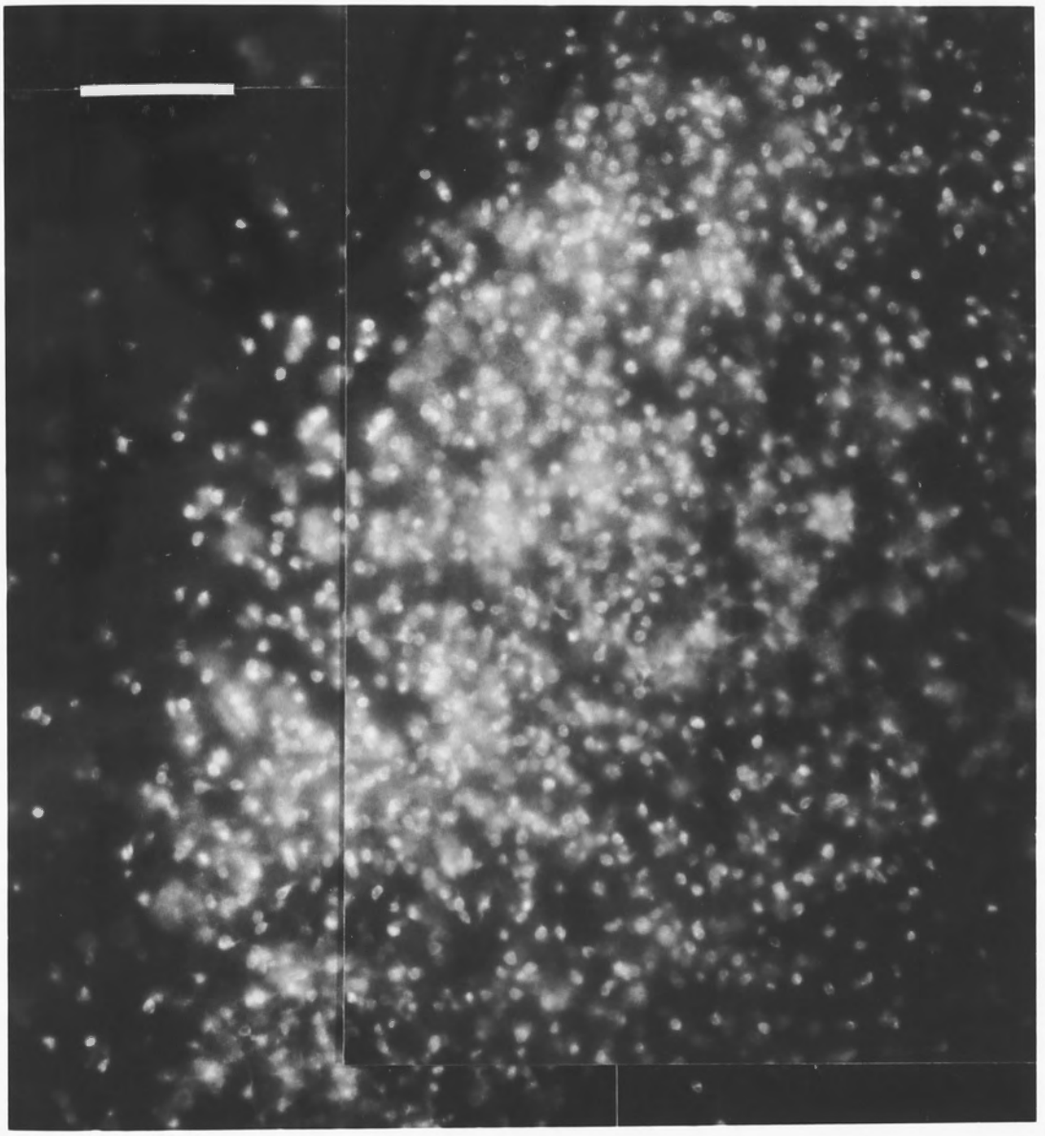


Figure 3.18 Photomontage of fluorescent image of the distribution of retinal ganglion cells projecting ipsilaterally to the SC at 28 days

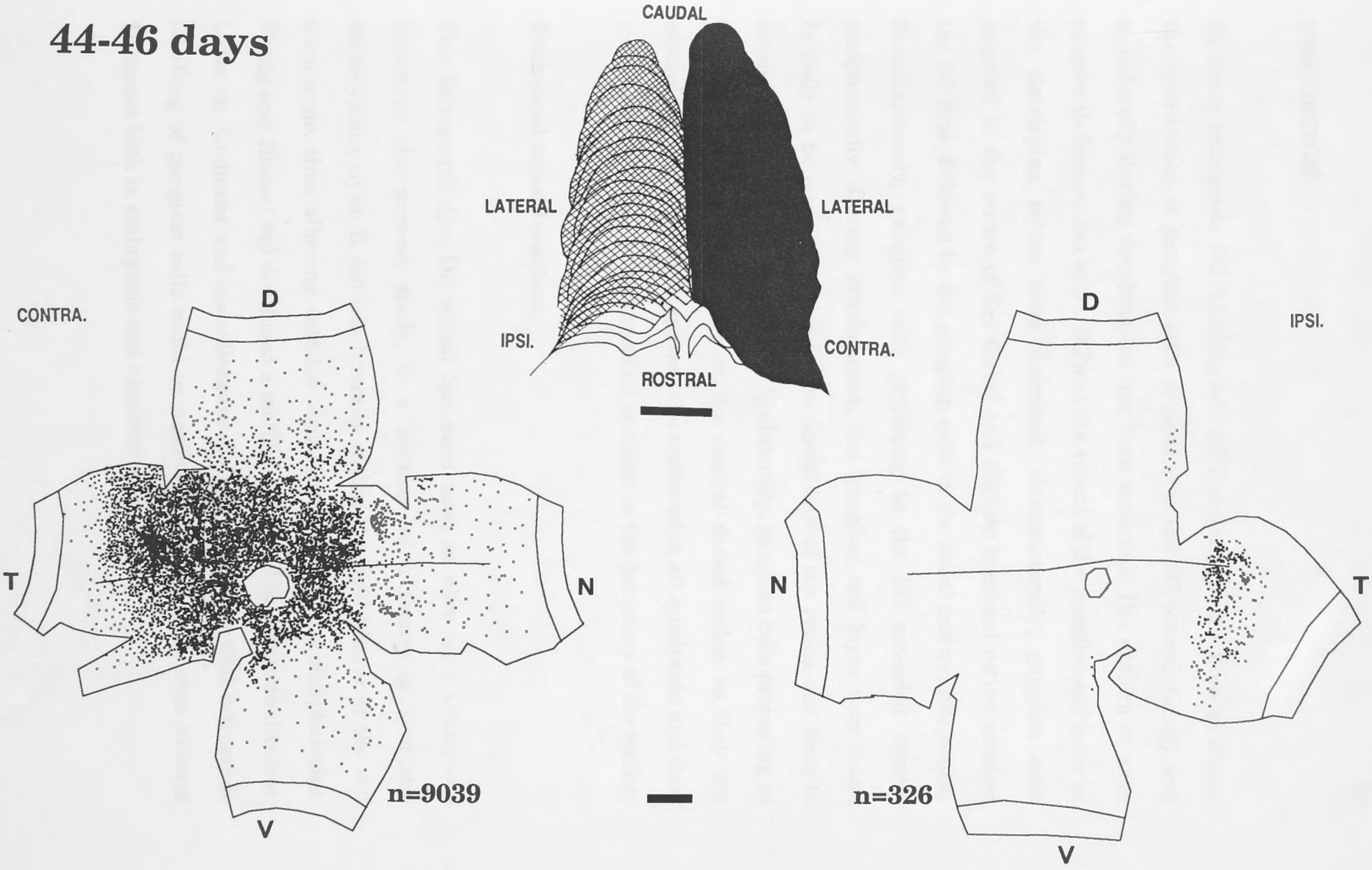
Ganglion cells labelled by DiI ipsilateral to the SC within the crescent-shaped region in the periphery of the temporoventral retina. Bar: 100 μm.

Figure 3.19 Camera lucida drawings of wholemounts of the retina and the reconstructed SC showing the position of the retinal ganglion cells projecting to the SC and the retinal innervation to the SC at 44-46 days

Conventions are the same as for figure 3.4 and 3.5. In addition, the line crossing temporonasally in the retina is the pigment line which indicates the border of light (upper) and heavy (lower) pigmentation. The distribution of ganglion cells contralaterally is qualitatively similar to that at earlier stage. Many ganglion cells are spread contralaterally over almost the whole retina. The density of labelled cells is higher more centrally, mainly in the temporodorsal retina and gradually falls off peripherally. The visual streak can be recognized in a band temporonasally around optic disc. Labelled cells are restricted ipsilaterally in a distinct crescent in the periphery of temporoventral retina. For the first time, retinal axons are found to be distributed throughout the entire rostrocaudal and lateromedial extent of the contralateral and ipsilateral SC. Bars: 1 mm.

CAUDAL

44-46 days


CAUDAL
LATERAL
LATERAL
IPSI.
CONTRA.
ROSTRAL
CONTRA.
D
T
N
V
n=9039
D
IPSI.
N
T
V
n=326

DISCUSSION

By using retrograde DiI labelling and CFV staining in the present study, the distribution of ganglion cells projecting to the SC contralaterally and ipsilaterally during development has been analysed. The pattern of axon outgrowth from retina to the SC and the extent of the ganglion cell layer in the developing retina were described. Contralaterally, ganglion cells situated in the centre of the dorsal and slightly temporal retina reached the SC first, followed by the ganglion cells in the nasal and ventral retina. Simultaneously, ganglion cells projecting to the SC extended centro-peripherically. During development, the ganglion cell layer was found initially to be more extensive in the dorsal half of the retina and then to extend ventrally and peripherally. Ipsilaterally, ganglion cells projecting to the SC were located initially in the central dorsal retina as they are contralaterally followed by sparse cells scattered in all quadrants and then were confined to the temporoventral crescent in the periphery of the retina.

Technical considerations

The fluorescent dye, DiI which has been used *in vitro* as a retrograde tracer in the present study, is a member of a family of synthetic carbocyanine dyes. It diffuses laterally within the lipid bilayer of the cell membrane, thus allowing detailed visualization of cellular morphologies. Honig and Hume ('86) developed a method to use DiI as a neuronal tracer. Later on, Godment and his colleagues ('87) demonstrated that retrograde labelling of ganglion cells could be achieved in fixed tissue over various distances both in embryonic and neonatal animals.

To correctly make the observation on the distribution of ganglion cells and the order of outgrowth of retinal ganglion cells projecting to the SC, it is important that all retrogradely labelled ganglion cells in the retina arise only from the SC. That is, those ganglion cells only projecting to the SC but not to the lateral geniculate nucleus (LGN) were labelled. That this is the case is supported by two pieces of evidence. First, as the location of DiI crystals in the SC was examined in each case or DiI-labelling was scanned in transverse sections of the SC, ectopic DiI crystals were not detected in any of the cases, and the fluorescence in the SC and the retrogradely labelled axons in the optic tract was much brighter than those bordering the deposit site. It is unlikely that this diffusion of DiI outside the SC effectively labelled ganglion cells in the retina. When a DiI deposit was made in both the SC and the LGN at 13 and 14 days (unpublished data from L.R. Marotte), the numbers of labelled retinal ganglion cells projecting to both the SC and the LGN were higher than those projecting only to the SC at the same age in the present study. The labelled ganglion cells in the retina contralateral to the DiI deposit were also distributed much more extensively in retina, particularly in ventral retina, than those in the present study. The data from Marotte also showed that an adult-like pattern of distribution of ganglion cells with uncrossed axons had already appeared at 13 days, 3 days earlier than the finding in the present study, with the majority of labelled ganglion cells located in the periphery of the temporoventral retina. This earlier appearance of the adult-like pattern presumably correlates with the earlier retinal innervation of the LGN, which was found by using an anterograde tracing technique (personal observation). In contrast, in the present study, a concentration of cells in the temporoventral retina did not appear until 16 days. Thus, it is quite convincing that only retinal ganglion cells which project to the SC were labelled retrogradely by DiI crystals placed in the SC.

In addition, the efficiency of retrograde transport of DiI from the SC is also crucial in making correct observations in the present study. In order to obtain reliable results in cell-distribution experiments, it is necessary to have good labelling efficiency over the entire retinal region. Some reassurance that this was achieved in the present experiments was obtained by comparing the distribution of ganglion cells retrogradely labelled by DiI with the distribution of ganglion cells stained by CFV. At the same developmental stage, the distribution of ganglion cells labelled by DiI or stained by CFV generally covered a similar region and percentage of retinal area, although a different distribution was found initially at 4-5 days. This finding could be explained by ganglion cells sending an axon into the brain a short time after differentiating. Although no evidence directly showed that every ganglion cell projecting to the SC was labelled, the findings suggest that at least, labelled ganglion cells arise from the entire region of the ganglion cell layer in the retina which has developed.

The extension of retinal ganglion cells projecting to the SC in the developing wallaby

Contralaterally. The ganglion cells entering the SC were first labelled by DiI in the central part of the dorsal and slightly temporal retina at 4 days. Subsequently at 9 days, the number of ganglion cells labelled retrogradely increased nasally and ventrally as well as dorsally and temporally, with the cells extending to more peripheral retina. Later on at 11-15 days, ganglion cells increased in numbers but showed a qualitatively similar pattern of distribution to that at earlier stages. By 16 days, the first hint of a visual streak and area centralis in the retina was seen contralaterally. Ganglion cells were positioned across the temporonasal pole,

predominantly in temporal and dorsal retina, with lower numbers extending into the nasal retina. At 28-32 days, numbers of ganglion cells projecting to the SC reached a peak, with a qualitatively similar distribution to that seen at younger stages. From 41-46 days, a dramatic fall in the numbers of labelled ganglion cells in the retina was found although the distribution pattern of ganglion cells innervating the contralateral SC remained similar to that seen at earlier ages. Observations on the distribution of ganglion cells stained by cresyl fast violet at the same age demonstrated a similar progress in extension of the ganglion cell layer during development. Ganglion cells were distributed initially across the central region of temporal and nasal retina and extended close to the periphery dorsally. Gradually, they spread over almost the whole retina, which correlated chronologically with the increase of the area covered by DiI-labelled ganglion cells.

Ipsilaterally. Ganglion cells in the retina ipsilateral to the SC were first labelled a day, at most, later than those contralaterally. The innervation of retinal axons arose from the same dorsal and central region of retina that gives rise to the initial contralateral projection. At 9 days, more ganglion cells found to project to the ipsilateral SC, were distributed diffusely in the retina. At 11-15 days, more labelled cells were situated relatively extensively over the retina, mainly in the dorsal part, with the periphery remaining devoid of labelling. As early as 16 days, a distribution pattern seen in adult mammals began to appear, in which ganglion cells were concentrated in a crescent-shaped region in the periphery of the temporoventral retina. A few scattered cells could be seen outside this region. At later developmental ages from 28 to 32 days, this pattern of distribution remains unchanged, although ganglion cells quantitatively increased. From 41-46 days, no cells were seen outside the temporoventral

retina and there was a large reduction in numbers of ganglion cells in this temporoventral region.

Topographic order of retinocollicular axons

A precise retinotopic order in the contralateral SC has been found anatomically and electrophysiologically in adult wallaby. Temporal retina projects rostrally, nasal retina caudally, dorsal retina laterally and ventral retina medially (Flett et al., '88; Mark et al., '93a). Ipsilaterally, the retinal projection to the adult SC appears as a series of discrete clusters confined to the rostral half of the SC but responses from the ipsilateral eye can not be detected eletrophysiologically (Wye-Dvorak, '84; Mark et al., '93a). In the present study on developing animals, it is clear that the dorsal and temporal retina projects to the contralateral SC first, followed by the nasal retina. The ventral retina is the last part to reach the SC. This complements the observations in chapter 2 and indicates that the earliest projection comes from roughly topographically appropriate parts of the retina. From 4 days after birth when the retinal axons first reach the rostrolateral edge of the contralateral SC, the ganglion cells, presumably extending these axons, are situated in the temporodorsal retina. As more ganglion cells, which are spread mainly throughout the temporal, dorsal and nasal retina, appear by 9 days, axons expand rapidly to cover the rostral-caudal extent of the SC but only in the lateral half. The extension of innervation caudally correlates with the increased numbers of ganglion cells in the nasal retina. As the innervation reaches the medial edge of the SC by 26 days, the numbers of cells in the ventral retina increase. The initial retinal projections to the contralateral SC come from regions of the retina topographically appropriate for the regions of the SC they are distributed on. The finding suggests that the contralateral retinocollicular

projection may be topographically organized from the time of its initial ingrowth, the first week after birth. In comparison with the contralateral projection, axons which arise from the similar central dorsal region of the retina, first reach the rostrolateral edge of the ipsilateral SC a day, at most, later. As axons spread to cover the region in the rostrolateral SC, ganglion cells supplying this projection are initially sparsely scattered in all retinal quadrants and then by 16 days are largely concentrated peripherally in the temporoventral retina. At 41-46 days, the projection covers the whole of the ipsilateral SC although all labelled cells are already concentrated in the crescent-shaped region in the temporoventral retina, showing the adult-like pattern. This organization of the retinal projection to the ipsilateral SC is puzzling. Early in development when ganglion cells projecting to the SC are scattered diffusely over the retina, the retinal innervation, confined to the rostroventral SC, does not arise from the topographically appropriate region of the retina. Later on when a concentration of cells in the temporoventral crescent apprears, the retinal projection covers the whole SC. In the adult the patchy projection is restricted in the rostral half of the ipsilateral SC and the ganglion cells supplying this innervation do not come from the topographically appropriate retinal region. One would expect that axons should be derived from dorsotemporal retina as well temporoventrally. It appears that cues governing the projection in the SC from the ipsilateral eye are imprecise. This finding, combined with the finding that physical function was not detected electrophysiologically (Mark et al., '93a), suggests that the ipsilateral projection in the wallaby may be vestigial.

Establishment of the distribution pattern of ganglion cells with crossed and uncrossed axons in other species

Studies on the sequence of ganglion cell outgrowth with crossed and uncrossed axons have also been carried out in other mammals, such as rodents. A similar pattern to that seen in the wallaby was demonstrated. In embryonic development axon outgrowth from the temporal portion of the retinal ganglion cell layer in hamster was detected prior to that in nasal retina (Jhaveri et al., '83). It was also shown in hamster that the retina contralateral to the HRP placement is non-uniformly labelled, and temporal retina is more densely labelled than nasal retina (Wikler et al., '85), as was found in the wallaby. It was suggested that temporal retina is preferentially represented as a result of either earlier axon outgrowth from this region or the earlier maturation of its appropriate target or both. Evidence obtained from developing quokka wallaby showed a process of maturation of ganglion cell distribution, in which ganglion cells are first generated in central retina, followed by those at increasingly peripheral locations (Harman and Beazley, '89). This finding is correlated with the finding in the tammar wallaby in the present study, where contralaterally projecting ganglion cells extend centro-peripherally with time. However, the ipsilateral projection in the hamster arises from the temporal periphery of the retina simultaneously with the contralateral projection in early development (Wikler et al., '85), which is different from that seen in the wallaby. In the wallaby, ganglion cells with uncrossed axons are distributed initially in the central dorsal retina followed later by the majority of cells being positioned in the crescent region of temporoventral retina.

The retinal origin of ganglion cells with crossed and uncrossed axons was demonstrated by using DiI retrogradely in mouse (Godement et al., '87; Colello and Guillery, '90). The sequence of outgrowth of ganglion cells projecting to the SC showed a similar pattern to that seen in the developing wallaby. Retinal ganglion cells contralateral to the dye placement site are labelled initially around the optic disc, mainly in the dorsal retina. During development, the area containing labelled ganglion cells in the contralateral eye enlarges gradually and the labelled region in the contralateral retina extends to all but the most peripheral margin. The ganglion cells projecting contralaterally in the mouse develop in a crude concentric fashion with earliest projecting cells in central and later ones in peripheral retina, which correlated with thymidine studies showing that retinal ganglion cells are generated in a rough central-to-peripheral gradient with oldest cells in central retina and the younger cells peripherally (Drager, '85; Godement et al., '87; Colello and Guillery, '90). Ipsilaterally, as in the wallaby, the earliest retinal projection in mouse arises mainly from the central dorsal retina, and, also as in the wallaby, is followed soon after by the adult-like distribution pattern, in which ganglion cells are concentrated mainly in the periphery of the temporoventral retina.

Other species display a similar refinement of the ipsilaterally projecting ganglion cells to that seen in the developing wallaby. In developing ferrets, it was reported that the majority of the ganglion cells projecting to the ipsilateral SC are dominant in the temporal crescent relatively early in development, with a number of cells scattered across the rest of the retina which are largely eliminated during development (Henderson et al., '88; Thompson and Morgan, '93). In cat, it also appears that ganglion cells projecting ipsilaterally are confined to the temporal retina early on, and

this is obvious prior to the later loss of ganglion cells (Williams et al., '86; Lia et al., '87). Similarly in rodents early in development, most of the uncrossed ganglion cells are concentrated in the temporoventral crescent-shaped region of the retina and those cells outside the region are seen to be preferentially eliminated later in development (Martin et al., '83; Jeffery, '84; Insausti et al., '84). However, the monkey (Chalupa and Lia, '91) shows a precise definition of the partial decussation line of ganglion cells early in development. At later fetal stages, no obvious refinement of ganglion cells projecting to the SC was found.

A different early distribution pattern of contralaterally projecting ganglion cells, which was not reported in the wallaby, was shown in ferret (Baker and Reese, '93). At the time when a concentration of ipsilaterally projecting cells is first seen in temporoventral retina, the distribution of retrogradely labelled cells in the contralateral retina falls off precipitously in this region. That is, the distribution of the cells in the temporoventral crescent of the ipsilateral retina initially occupies a further eccentric location than does the distribution of contralaterally projecting cells. This finding was also demonstrated in mouse (Drager, '85) and rat (Bunt et al., '83). The discrepancies between these findings in ferret (Baker and Reese, '93), mouse (Drager, '85) and rat (Bunt et al., '83) and in the wallaby could be that the ganglion cells examined in the wallaby and in the other studies were projecting to different targets. The results that found the initial position of temporal labelling in the ipsilateral retina is further eccentric than that in the contralateral retina are obtained from ganglion cells projecting to the LGN as well as the SC. Ganglion cells projecting to the LGN are likely to be labelled retrogradely earlier than those projecting to the SC, as the LGN is innervated earlier than the SC. Thus, the pattern found in the ferret, mouse and rat may represent the early projection to the

LGN. However, this does not explain the discrepancy between the results of Drager ('85) and Collelo & Cuillery ('90) for the mouse. The latter study obtained similar results to those obtained in the wallaby, even though in both studies in the mouse ganglion cells projecting ipsilaterally are labelled retrogradely both from the LGN and the SC.

Axonal outgrowth from the retina to the tectum has also been characterized in non-mammalian vertebrates. In frog (Holt, '84), ganglion cells from the dorsal retina leave the eye first, followed later on by those from the ventral retina. It was also reported in chick (McLoon, '85) that ganglion cells giving rise to the first retinal axons to enter the tectum were identified in the central retina, dorsal-nasal to the optic fissure.

Possible mechanisms to establish the distribution of contralaterally and ipsilaterally projecting ganglion cells

The organization of the early distribution of ganglion cells projecting to the SC raises a question: what mechanisms are responsible for the initial establishment. It is clear from the present study in the wallaby that the distribution pattern of ganglion cells with crossed axons extending over the whole retina with a visual streak and the cells with uncrossed axons concentrated primarily in the crescent-shaped region in the periphery of the temporoventral retina, is established very early in development. This distribution appears prior to the start of cell death, which in the wallaby begins about 30 days after birth (Spira and Marotte, '89). This finding is consistent with the studies in other mammals such as mouse (Drager, '85; Godement et al., '87; Sretavan, '90; Colello and Guillery, '90) and ferret (Baker and Reese, '93; Thompson and Morgan, '93). The observations suggest that the adultlike decussation pattern of a large number of the

ipsilaterally projecting ganglion cell is established as the retinal ganglion cells approach the optic chiasm during their initial outgrowth from the eye. It has been postulated that specific routing of retinal ganglion cell axons at the optic chiasm is due to the presence of specific local guidance cues during early development (Beazley, '75; Guillery and Casagrande, '76; Sretavan, '90; Cucchiaro, '91; Baker and Reese, '93; Sretavan and Reichardt, '93). For example, in the mouse, topography of retinal axons in the optic nerve as they appoached the chiasm, combined with local environment factors such as a knot-like glial formation, was postulated to play an important role in guiding axons at the chiasm (Silver, '84). However, Colello and Guillery ('90) reported that there is no clear segregation of ipsilaterally projecting retinal axons in the optic nerve at any stage of development. The uncrossed axons coming from the temporoventral crescent of retina lie in the corresponding region in the optic nerve just behind the eye but then disperse to occupy the whole cross-sectional area along the length of the optic nerve. It was consequently concluded that the cues that act to send one group of retinal axons ipsilaterally and another contralaterally cannot be dependent upon the position of the axons in the pathway. More recent studies have suggested that these cues may be molecular in nature. Studies in mouse indicated that the chiasmatic midline exerts an inhibitory effect upon temporal, but not nasal, retinal ganglion cell axons as they invade the chiasm during development (Godement and Mason, '90; Godement et al., '90; Mason et al. '90; Sretavan, '90; Guillaume et al., '91).

It has also been suggested that the time of axonal arrival at the optic chiasm determines the side of the brain to which retinal ganglion cells will project. In mouse (Drager, '85), rat (Reese and Colello, '92) and cat (Reese et al., '92), the time of genesis of ganglion cells in the temporal retina has

been postulated to govern the distribution pattern of retinal ganglion cells projecting contralaterally and ipsilaterally at the chiasmatic region. Earlier generated cells all had nondecussating axons while later generated cells had axons that were more likely to project ipsilaterally and contralaterally. Together, these studies strongly support the hypothesis that the different distribution pattern of ganglion cells projecting contralaterally and ipsilaterally is primarily established as their axons navigate through the chiasm. However, in these studies, the very first axons from retinal ganglion cells, mainly in the dorsal retina which initially project both ipsilaterally and contralaterally were not demonstrated. These initially ipsilaterally projecting cells reported in the wallaby and mouse (Godement et al., '87; Colello and Guillery, '90), would be eliminated by cell death later on.

In addition, the contribution to the adult pattern of retinal ganglion cell distribution made by ganglion cell loss was also reported. Although selective cell death may refine the decussation pattern of ganglion cells, such regressive events are not the primary mechanism responsible for the creation of the distribution of ganglion cells (Jeffery and Perry, '82; Bunt et al., '83; Martin et al., '83; Insausti et al., '84; Jeffery, '84; Godement et al., '87; Leventhal et al., '88; Sretavan, '90; Colello and Guillery, '90). As mentioned previously this also appears to be the case in the wallaby. There was a large decrease in the number of labelled ganglion cells between days 28 and 46 but the pattern both contralaterally and ipsilaterally was established prior to this time. This loss was likely to be due to cell death as it is during the period when dying cells are seen in the ganglion cell layer (Spira and Marotte, '89) but could also include retraction of collaterals by retino-thalamic axons. It is unlikely that this

was an artifact of incomplete labelling as the transport time for DiI was lengthened considerably between these two ages.

SUMMARY

It is clear, in the present study, that axons of ganglion cells in dorsotemporal retina reach the contralateral SC first, followed by those from nasal and ventral retina, with the region of retina projecting to the SC extending centro-peripherally. The ipsilaterally projecting ganglion cells, located initially in the central dorsal retina, become restricted to the periphery of the temporoventral retina relatively early in development. These findings complemented by the findings in chapter 2, suggest that the retinal projection, at least to the contralateral SC, may be topographically organized as it grows into the SC. The nature of the organization of the ipsilateral projection is less predictable. However, more precise detail on the degree of retinotopic organization in the contralateral and ipsilateral SC could not be inferred from these results. For this, information is needed on the precise distribution of axons in the SC from individiual retinal quadrants during development. This is investigated in the next chapter.

Chapter 4. The Development Of Topography In The Retinal Projection To The Superior Colliculus

INTRODUCTION

The findings in chapter 2 and 3 suggest that the contralateral retinocollicular projections appear to be roughly topographically formed from the time of their initial ingrowth, the first week after birth. From 4 days after birth when the retinal axons first reached the rostrolateral edge of the contralateral SC, the ganglion cells extending these axons were situated in the appropriate topographic region, the temporodorsal retina. As more ganglion cells, which were spread mainly throughout the temporal, dorsal and nasal retina, appeared by 8-9 days, axons expanded rapidly to almost cover the rostral-caudal extent of the SC but only in the lateral half. Subsequently, the extension of innervation caudally correlated with the increased numbers of ganglion cells in the nasal retina. As the innervation reached the medial edge of the SC by 26 days, the numbers of cells in the ventral retina increased. In comparison with the contralateral projection, the organization of the ipsilateral projection seems less predictable. Ipsilateral axons first reached the rostrolateral edge of the ipsilateral SC a day later and arose from the same temporodorsal retina as those contralaterally. They then spread to cover the region in the rostrolateral SC, but ganglion cells supplying this projection were distributed over the retina. When the ganglion cells projecting to the ipsilateral SC were concentrated in the peripheral temporoventral retina from 16 days, the retinal projection gradually covered the whole of the ipsilateral SC. Thus, information on the distribution of individual retinal axons in the SC during development of the retinocollicular map in the

wallaby remains to be given. This is crucial to an understanding of the formation of specific visual neuronal connections.

Studies of establishment of order in the retinotectal projection have been carried out mainly in non-mammalian vertebrates. During development of retinotectal projections in fish (Stuermer, '88a; Stuermer and Raymond,'89; Kaethner and Stuermer,'92) and frogs (Holt and Harris, '83; Holt, '84; Sakaguchi and Murphey, '85; O'Rourke and Fraser,'86, '90; Fujisawa, '87), a degree of topographic organization is present from the beginning of the development. There is a significant increase in the precision of the visual organization, this occurs slowly and results from a disparate growth of the tectum relative to the size of retinal arbors, rather than by large-scale pruning of arbors or eliminating of mistargeted axons. Studies of the developing chick retinotectal projection (McLoon, '82; Nakamura and O'Leary, '89) suggest that the retinal ganglion cells initially project diffusely to the tectum and that the topographically aberrant projections are eliminated during subsequent development. However, another study on the axonal arborization in the developing chick retinotectal system (Thanos and Bonhoeffer, '87) suggest that most retinal axons grow more or less directly to their projection target field along straight routes.

Studies on the regeneration of the visual pathway in frogs and fish (Fujisawa, '81; Fujisawa et al., '82; Cook, '83; Stuermer, '88b,c) add to understanding of the formation of topographic connections. During regeneration, retinotopic order emerges from an initially diffuse projection before the regenerating axons make terminal arbors at their retinotopically correct site.

Less is known of the topographic arrangement in the mammalian retinocollicular system during development, since such study has been limited by the techniques available for axon labelling during the embryonic stage. In the main, studies done were carried out on rats. Investigations with anatomical (Lashley, '34) or electrophysiological methods (Siminoff et al., '66), confirmed that, in mature rats, the retinal projection to the contralateral SC is precisely ordered in a topographic fashion. The ipsilateral projection is extremely sparse (Lund, '65) and cannot be detected electrophysiologically (Simonoff et al., '66; Fukuda et al., '84). Observations on the topographic organization of neuronal connections in developing rats have also been made by retrograde and anterograde tracing techniques. With injections of retrograde tracers into the SC of neonatal rats, the labelled ganglion cells were observed in topographically appropriate parts as well as in topographically inappropriate parts of the contralateral retina (O'Leary et al., '86) and ipsilateral retina (Martin et al., '83). A later investigation (Yhip and Kirby, '90), using a similar retrograde method with fluorescence tracer in the SC of neonatal rats, showed that the location of the majority of labelled ganglion cells observed was in an appropriate topographic region in the retina, although some errors in topographic projection were found. In addition, Simon and O'Leary, in a series of investigations ('90, '91, '92a,b,c), used an anterograde tracing method to label axons from different retinal quadrants in prenatal and neonatal rats. Their studies revealed that developing retinal axons widely mistarget and grow over most of the contralateral SC. However, they also found that there was a preference for axons to arborize in the topographically appropriate regions of the SC, regions which are considerably larger than those at later stages. Thus, their findings indicated a limited specificity in the retinal topographic targeting and branching during early development.

In common with rats and other mammals, an adult marsupial wallaby such as the adult tammar was found anatomically and electrophysiologically to have a precise retinotopically organized retinal projection to the contralateral SC (Flett et al., '88; Mark et al., '93a), while the ipsilateral projection appeared as a series of discrete clusters confined to the rostral half of the SC and responses from the ipsilateral eye could not be detected electrophysiologically (Wye-Dvorak, '84; Mark et al., '93a). Marsupials are born at an extremely early stage of neural development before the fibres of the optic nerve make any connections with the brain. The tammar has been used as a model to study the development of retinotopy in projections from the eye to the SC and to work out the mechanisms controlling the formation of orderly nerve patterns. In an anterograde labelling study (Marotte, '90), small retinal lesions were made and the remaining retinal projections were traced with the enzyme horseradish peroxidase (HRP). The results showed that, from 43 to 93 days after birth, there was an increase in precision of the retinotopy as judged by an increase in sharpness of the border of filling defects in the projection labelled with HRP. It was concluded that a less precise retinal projection can be observed in the SC at 43 days after birth and that the refinement in the projection was complete around 93 days prior to eye opening. However, prior to 43 days, no retinotopy could be observed using this technique. Marotte (93'), using a retrograde tracer in the caudal SC, also reported that by 92 days refinement of the projection, revealed as a loss of inappropriately projecting ganglion cells, was complete. In this study, however, ganglion cell axons were initially found to be distributed on the contralateral SC in a coarse retinotopy from at least 30 days onwards, when a dense patch of retrogradely labelled ganglion cells was detected to be appropriately positioned in the proper retinal region, although

accompanied by inappropriately positioned labelled cells elsewhere. This is earlier than found in the previous study using retinal lesion and anterograde labelling (Marotte, '90)

In the present study, a more direct and more sensitive method was used to determine how retinocollicular projections are topographically formed from the initial stage of development onwards. This involved using the fluorescent lipophilic carbocyanine dye 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) as an anterograde tracer *in vivo* to label small groups of axons in the retina, either in the periphery or more central regions of the retina. Tracer was placed in each of the four retinal quadrants of pouch young wallabies at ages ranging from 8 days, when there is a reasonable number of retinal ganglion cells projecting mainly to the rostral and the lateral half of the SC, to 90-95 days, when as previous studies showed, a precise retinotopic projection is present. The sequence of events in the development of the retinotopic projection to the SC in developing animals will be described in detail.

MATERIALS AND METHODS

Animals

Sixty two pouch young wallabies (*Macropus eugenii*) aged 8 (n=1), 12-13 (n=4), 22-24 (n=6), 27-28 (n=13), 41-47 (n=11), 52-55 (n=7), 61-68 (n=12) and 90-95 (n=8) days were used in this study (Table 4.1-2). All animals aged 22 days or younger were of exactly known birth date while the age of older animals was determined from a chart of head lengths of animals of known age (W.E. Poole, personal communication) or from birth date.

Labelling axons

The animal was anaesthetised by either hypothermia or by intramuscular ketamine (Parke-Davis) and xylazine (Rompum, Bayer Australia). All animals younger than 55 days were anaesthetised by hypothermia. Older animals were anaesthetised by either hypothermia or intramuscular ketamine and xylazine. For animals aged 90 to 95 days, doses of 0.5 mg ketamine/100 g body weight and 0.1 mg xylazine/100 g body weight were used, and half this dose was used for younger animals.

After the eyelid was opened, a small hole was initially made by a glass pipette or scissors from behind the eye through sclera and retina. A small piece of gelfoam (about 0.1 mm in diameter, Upjohn) impregnated with DiI was then inserted into the hole and the eyelid was repositioned. The gelfoam with DiI was made by soaking a piece of gelfoam with a saturated solution of DiI in ethanol or with 8.2% solution of DiI in dimethylformamide. For easier application, the gelfoam were dried at room temperature before use. In one case, the same method as used in rat (Simon and O'Leary, '92a) was applied by injecting 0.05 µl of 8.2% solution of DiI in dimethylformamide into the periphery of the dorsal retina. Focal deposits of DiI were made into the periphery of each of the four retinal quadrants: temporal (n=16), nasal (n=19), dorsal (n=11) and ventral (n=11), in different age groups of animals: 8, 12-13, 22-24, 27-28, 41-47, 52-55, 61-68 and 90-95 days. In 5 cases aged 24, 28, and 90-95 days, deposits were made into more central retina (temporal, n=2; nasal, n=1; dorsal, n=1; ventral, n=1). The positioning of DiI deposits was made according to the alignment of the pigment line. This line was predicted using the method described in chapter 3, p47.

Animals recovered from anaesthesia in an artificial pouch, in an incubator at 37°C. Pouch young, 55 days or younger, were reattached to the teat. During this procedure the mother was anaesthetised intravenously through the tail vein by 2.5--4 ml of 2.5% Brietal Sodium (Lilly). Animals from 61 days were able to reattach to the teat by themselves when they were replaced in the mother's pouch.

Histology

After 2--3 days survival animals younger than 90 days were deeply anaesthetised by hypothermia while older ones were anaesthetised by 0.3 ml of 6% Pentobarbitone Sodium (Nembutal, Boehringer Ingelheim) intramuscularly. Animals were perfused transcardially with 0.9% saline briefly, followed by a fixative of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for twenty minutes. The whole head, after removal of the dorsal part of the skull and the cornea, was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for 2-3 hr or overnight. The retina, in which the inserts of DiI had been made, was dissected and a wholemount prepared on a glass slide. It was coverslipped using anti-fade buffer as a medium: 2% n-propyl gallate and 80% glycerol in 0.1 M phosphate buffer, pH 7.4. In addition, the entire SC, both contralateral and ipsilateral to the eye with the DiI deposit, was dissected under a surgical microscope and mounted on a special concave slide which protected against damage to the tissue from the cover slip since the whole mount of the SC was thick. The mounting medium described above was used. The wholemounts of retina and the SC were then examined in a Leitz fluorescence microscope with an excitation filter of M2, Bp546/14. Some colliculi labelled by DiI in the ventral retina, at 90-95 days, were later embedded in an albumin gelatin mixture hardened with glutaraldehyde (chapter 2, p26) and sectioned

sagittally at 200-250 μm on a vibratome. Every section was mounted serially on glass slides with antifade buffer as mentioned above and observed in the fluorescence microscope.

Analysis

The NeuroTrace computer system combined with a Leitz fluorescence microscope (chapter 3, p48) was also utilized to record and analyze the observations from the wholemounts of retina and the SC in the present study. In the observations of retina, the outline of the wholemount was drawn first. The size of the DiI placement and labelled axons between the site of DiI and the optic disc was then added to this outline. In the observations of the SC, individually labelled retinal ganglion cell axons were traced, those tipped with growth cones and the position of any terminal zones were marked. In some cases every labelled axon was drawn. In other cases, this was not feasible because of the large numbers of overlapping axons labelled by DiI deposit. In these cases, representative axons and any growth cones within the labelled area and those axons defining the rostrocaudal and mediolateral extent of this area, as well as any solitary labelled axons outside the area, were recorded. This NeuroTrace program provided a rapid, direct and detailed recording method for the present study.

Following the reconstruction of the retina and the SC, the area of whole mounts and the labelled area of DiI deposits in retina and the area covered by labelled axons and terminal zones in the contralateral SC were measured by using an IBM compatible computer imaging system with the scientific measurement program, Sigma-Scan version 3.0 (Jandel Scientific). The percentage of retina covered by the DiI deposit, and the

percentage of the SC covered by labelled axons and terminal zones were then calculated.

The amount of axonal branching was quantified in two groups of animals aged 27-28 (n=3) and 52-55 (n=3) days, in which deposits of DiI were made into the periphery of the nasal retina. Wholemounts of the SC were observed at 250 x in a Leitz fluorescence microscope. In each animal, twenty individual labelled nasal axons were chosen as the minimum number for branch counting, in the rostral and caudal half of the SC, separately. Every side branch formed from these axons was counted. Mostly, axons could be traced from rostral to caudal and the counting in the rostral and caudal half was performed on the same axon. When axons could not be traced over this distance, only axons which could be traced over either the entire rostral or entire caudal half of the SC were chosen.

In some cases aged 12, 22-24, and 28 days, selected growth cones were observed and photographed using a laser scanning confocal microscope (MRC 500, Bio-Rad with a Nikon fluorescence microscope). The confocal microscope created an optical section, allowing the visualization of one focal plane of a fluorescent specimen without the interference of scattered light from outside the plane of focus (White et al., '87; Fine et al., '88)

RESULTS

All the tables and figures for this chapter are grouped together at the end of the results section.

DiI placement in the retina

The organization of the retinotopic projection to the contralateral SC in the mature wallaby obeys the common non-primate mammalian plan, with temporal retina projecting rostrally, nasal retina caudally, dorsal retina laterally and ventral retina medially (Flett et al., '88; Mark et al., '93a). To characterize the emergence of topographic order in the retinocollicular projection, a focal DiI placement in developing animals was made peripherally or more centrally into these particular regions, each of the quadrants in temporal, nasal, dorsal and ventral retina. A typical placement of DiI in the periphery of retina is shown in Fig.4.1A and B. A focal DiI deposit was found in the periphery of retina. Labelled ganglion cell axons formed a bundle which could be followed from the deposit site to the optic disc. As they entered the optic disc, labelled axons gathered together and then exited the eye. These axons travelled mostly in one compact bundle along the intraretinal course with a few exceptions in which axons were followed from deposit site to the optic disc in two bundles (Fig.4.12B, 4.23A). All labelled axons that left the eye were observed to originate from a single identified DiI placement site when each retina was carefully scanned for ectopic labelling sites. In many of the older animals aged 61 to 95 days, labelled axons could not be followed from the deposit site to the optic disc although the ganglion cell terminals were demonstrated in their target, the SC. The size of the DiI placement ranged from 0.1% of retinal area at the smallest to 8.1% at the largest, with an average of 2.2% (n=61, SD [standard deviation] =±1.8).

Developing organization of the retinal projection in the SC

The distribution of labelled retinal ganglion cell projections, the location and morphology of growth cones, the numbers and the pattern of branching, and the arborization of terminals in developing animals were characterized in detail. The analysis focused on consistent differences in the topographic order and distribution of retinal axons, not the exact number of axons that were labelled. Injections of comparable size or location showed some variability in the numbers of labelled axons. The labelling described was in two forms. Label was in the form of identifiable fibres of about 1-2 μm diameter, traceable for long distances in the wholemount. In addition, label was in the form of diffusely fluorescent patches in which a multitude of extremely fine branches was orientated in every direction. The latter, in agreement with Simon and O'Leary ('92a), are called terminal zones. Data comes from different age groups of pouch young wallaby ranging in age from 8-13, 22-24, 27-28, 41-47, 52-55, 61-68 and 90-95 days (Table 4.1-4). The following descriptions begin with the oldest age group and then go back to the early development in chronological order.

90 -- 95 days (n=8, Table 4.1, 4.3)

At this time, the mature retinotopic order of the ganglion cell axons was formed in the contralateral SC, when discrete terminal zones were seen, covering an area of 0.12 mm^2 in the SC on average. In some cases, labelled axons were seen to enter the SC at the rostral pole and ran caudally to reach the terminal zones.

Temporal deposit (n=3) After a DiI deposit in the periphery of the temporal retina, a discrete terminal zone elongated along the medial-lateral axis was present in the most rostral contralateral SC. No labelled axons were distributed outside the terminal zone (Fig.4.2A). Fine spots filled this terminal region on a cloudy background. The border of the

terminal zone was sharp (Fig.4.4A). In the ipsilateral SC, no labelled axons or a terminal region were observed.

Nasal deposit (n=2) In one case, a terminal zone was positioned caudally with no axons distributed outside it, after DiI deposits in the nasal retina (Fig.4.2B). In comparison with this, in another case, many labelled axons were followed from the rostral pole to the terminal zone in the far caudal SC. The discrete terminal zone with a sharply defined border contained fine spots on a cloudy background. It is shown in Fig.4.4B. Ipsilaterally, no labelling was seen.

Dorsal deposit (n=1) After a DiI deposit in more central dorsal retina, a discrete terminal zone was found to be positioned in the lateral contralateral SC, with a few axons running from the rostral pole to it (Fig4.3A). The terminal zone was also filled with fine spots on a cloudy background (Fig.4.4C). No labelling was seen ipsilaterally.

Ventral deposit (n=2) Following a more central DiI placement in the ventral retina, a discrete terminal zone was formed at the medial border of the contralateral SC (Fig.4.3B). This terminal zone was characterized as a focal region with fine branching (Fig.4.4D). Labelled axons or terminal zones were not detected in the ipsilateral SC.

8 -- 13 days (n=4, Table 4.1)

From these early stages, labelled retinal ganglion cell axons were observed to be distributed in the SC in a rough topographic order. No signs of terminal zones were present.

Temporal deposit (n=2) At 8 days, a few labelled axons from the temporal retina were observed at the rostral edge of the contralateral SC, distributed in a very small region. By 12 days, more labelled axons arising from a DiI deposit in the temporal retina extended into the SC and were confined to the rostral half. No axons could be followed into the caudal SC. In mediolateral extent axons were distributed in a region covering approximately one third of the SC with no axons in the most lateral or medial region. The fibres contributed evenly throughout the whole labelled region, with only two axons outside the main region (Fig.4.5.A), and occupied an area of 16% of the SC. All labelled axons followed a rostral to caudal course through the SC and the adjacent axons tended to be aligned approximately in parallel (Fig.4.6). Growth cones were seen within the region where axons were distributed at all rostrocaudal levels. They were seen at the tips of elongating axons trunks (Fig.4.7) and the majority of them were large in size and had a complex morphology with lamellopodia and filopodia (Fig.4.8). Branches on individual axons were uncommon, and only one axon was found with a few small side branches shown in Fig.4.9. The short branches (<10 μm in length) were found along the parent axon. Ipsilaterally, a few labelled axons tipped with complex growth cones were distributed at the rostral edge of the SC (Fig.4.5A).

Nasal deposit (n=1) In contrast to axons from temporal retina, at 13 days, many axons from nasal retina extended across the rostral-caudal axis to reach the caudal half of the contralateral SC although they were yet to touch the far caudal margin. Comparably, in the previous study (chapter 2), retinal axons were yet to reach the caudal pole of the SC by 12 days, not doing so until 18 days. Due to the slightly ventral deposit, the nasal axons travelled more medially to reach the caudal SC, through the region which is a target of ventral retinal axons. Labelled axons ran primarily in a

rostrocaudal direction. Most of them were evenly distributed within a mediolaterally confined band, distributing over a region of 20% of the SC. A few axons lay laterally but adjacent to this region (Fig.4.5B). Axons tipped with growth cones, which had complex lamellopodia and filopodia (Fig.4.8), were distributed throughout the labelled region. Branches at either topographically appropriate regions in the caudal SC or inappropriate regions in the rostral SC were rare. Labelled axons were not detected in the ipsilateral SC.

Dorsal deposit (n=1) Axons labelled from the periphery of the dorsal retina were distributed in the SC over a lateral region of the rostral half of the SC by 12 days (Fig.4.5C). The dorsal axons were expected to reach the most lateral edge of the SC, as they are found there at later ages (see below), but at this young age, there was a difficulty in identifying the lateral edge of the SC. Extra tissue could be included in this wholemount of the SC. The area occupied by the axons covered approximately 18% of the SC. Many of the labelled axons ended in growth cones with lamellopodia and filopodia (Fig.4.8) and they were found throughout the rostrocaudal extent of the labelled region. Branching of individual axons was very rare. Sparse labelled axons were found in the lateral part of the ipsilateral SC, confined to the rostral region (Fig.4.5C).

Ventral deposit (n=1) Labelled axons arising from the periphery of the ventral retina were found to enter the medial part of the contralateral SC and reached the caudal SC although they did not extend right to the caudal pole at 12 days. The axons covered an area of 18% of the SC. As expected they did not reach the far medial edge as retinal axons do not reach the medial border until 26 days (chapter 2). No labelled axons were seen in the lateral two thirds of the SC (Fig.4.5D). Axons were aligned primarily

rostrocaudally. Axonal growth cones having lamellopodial extensions or filopodial characteristics (Fig.4.8) were found throughout the labelled regions. Few axons with side branches were seen. No labelling was detected in the ipsilateral SC.

22 -- 24 days (n=6, Table 4.1)

At this stage, labelled axons from each retinal quadrant were distributed in a coarse retinotopic order. No terminal zones were seen.

Temporal deposit (n=2) Labelled axons from a deposit of DiI in the temporal retina, were observed to be confined to a small region at the rostral contralateral SC, covering 14% of the collicular area. No axons were found outside the labelled region and the axons tipped with growth cones were distributed evenly over this region (Fig.4.10A). Most growth cones bore lamellopodia and filopodia, as did those in the younger animals (Fig.4.8). Branching of axons was still uncommon. In comparison to earlier stages, many labelled axons with growth cones covered a larger region in the ipsilateral rostral SC. The axons formed a mirror image to that seen contralaterally and were distributed evenly in the labelled region. Few branches could be detected (Fig.4.10A).

Nasal deposit (n=2) Labelled axons from the nasal retina were distributed in the contralateral SC, similar to those seen at earlier stages. They extended towards the far caudal pole, distributing over a region of 24% of the SC. Since the DiI deposit, which was placed in the nasal retina, extended slightly ventrally, the nasal axons were traced caudally along the medial border -- the target of ventral retinal axons (Fig.4.10B). Many axons with complex growth cones with lamellopodia and filopodia (Fig.4.8) were

seen uniformly in the labelled region. Branching of axons was very rare. Ipsilaterally, only one labelled axon from the nasal retina was observed at the rostral SC (Fig.4.10B).

Dorsal deposit (n=2) Retinal axons labelled by the gelfoam with DiI solution in the dorsal retina were distributed over the lateral part of the contralateral SC, with axons extending further caudally than in younger animals (Fig.4.10C). A similar pattern of projection of retinal axons labelled by focal injection of 8.2% DiI solution in dimethylformamide was also observed. This method was used to investigate the retinocollicular projections in rat (Simon and O'Leary, '92a) The axons covered an average area of 13% of the SC. Axonal growth cones with lamellopodia and filopodia were found throughout the labelled region (Fig.4.8). Branching, observed in the form of short side branches, was very rare. Only one axon extending for a very short distance was seen in the lateral ipsilateral SC (Fig.4.10C).

Ventral deposit (n=2) Labelled axons from the ventral retina extended contralaterally along the more medial SC in a narrow band, covering 14% of the collicular area. One labelled axon was found rostrally outside the band. The axons still did not reach the medial border (Fig.4.10D). Anterograde tracing of retinal axons with HRP (chapter 2) also showed that the retinal projection did not extend to the medial edge at this age. The observations on the growth cones and branching of axons were similar to those seen at earlier stages. Ipsilaterally, a few labelled axons from the ventral retina were followed in medial SC in a rostrocaudal direction but were confined to the rostral part of the SC (Fig.4.10D).

27 -- 28 days (n=13, Table 4.1)

At this age, axons were distributed in a rough topographic order similar to that seen at 22-24 days.

Temporal deposit (n=3) In two animals, DiI deposits were placed in the periphery of the temporal retina, labelled axons in both cases contributed evenly in the rostral contralateral SC and covered an area of 18% of the colliculus, without significant branching. The results of one of these animals is shown in Fig.4.11A. Axons bearing complex growth cones with lamellopodia and filopodia (Fig.4.8) were distributed throughout the labelled area. Labelled axons from a more central deposit of DiI in the temporal retina were also distributed rostrally with a coverage of 22% of the SC (Fig.4.11B). The behaviour of retinocollicular projections, branching and growth cones was similar to that seen from the peripheral labelling. In the ipsilateral SC, many labelled axons from both peripheral and more central deposits, were confined to regions in the rostral SC. These ipsilateral projections were more or less mirror images of the contralateral ones about the medial axis (Fig.4.11A,B).

Nasal deposit (n=5) In three cases, axons labelled by DiI in the nasal and slightly ventral retina were followed rostrocaudally in the contralateral SC along the medial border, with the labelled axons reaching the caudal pole. The axons were aligned rostrocaudally in parallel. One example is given in Fig.4.12A, in which one stray axon was seen more laterally. In one case (Fig.4.12B, 4.13), when the nasal axons were followed from a DiI placement to the optic disc in two compact bundles, more dorsally and more ventrally, the labelled axons in the SC were traced rostrocaudally throughout the whole mediolateral extent of the

contralateral SC, with axons gathering together at the caudal pole. On average, the area covered by the axons from the periphery of the nasal retina was 48% in the SC. In another case, labelled axons from a more central DiI deposit in nasal retina showed a similar distribution to that from the peripheral deposits, with axons running from the rostral to the caudal pole in the SC. In each case, many axons with complex growth cones (Fig.4.8) were observed to be distributed at all rostrocaudal levels of the SC.

To analyse branching of axons quantitatively, branches of the individual nasal axons in three animals were counted in the rostral and the caudal half of the SC, respectively (see Materials and Methods). Examples of branches are shown in Fig.4.14. At this stage, 11.6% (n=3, mean=11.6%, SD=±1.7, total axons=60) of labelled axons in the rostral SC had only one side branch of 20 μm or less in length. The same degree of branching was seen in the caudal SC (n=3, mean=11.6%, SD=±1.7, total axons=60). Ipsilaterally, no labelled axons from the nasal retina could be detected in any of the cases.

Dorsal deposit (n=2) Retinal axons labelled by DiI in the dorsal retina were distributed similarly to younger ones, in a region confined to the lateral contralateral SC. The area in the SC covered by the axons was 13% on average (Fig.4.15A). Morphology of growth cones was complex (Fig.4.8) and branching of axons was uncommon. Only one labelled axon was observed laterally in the ipsilateral SC. It did not extend into the caudal SC (Fig.4.15A).

Ventral deposit (n=3) Axons labelled by DiI in the periphery of the ventral retina extended from the rostral to caudal pole along the medial

border of the SC. By this age, they ran along the far medial border (Fig.4.15B). This finding coincided with the finding in chapter 2: The contralateral projection of retinal ganglion cells first reached the far border of the medial SC by 26 days. The axons were aligned rostrocaudally in parallel (Fig.4.16). The coverage by the labelled axons in the SC was 28% on average. Many axons with growth cones were present throughout the labelled area at all rostrocaudal locations. They had a complex morphology as previously described (Fig.4.8). Axonal branching was rarely seen. Ipsilaterally labelled axons from the ventral retina were distributed rostrocaudally in the medial region of the SC. The projection was a mirror image of the contralateral one around the medial border of the SC, although the ipsilateral projection did not reach the far caudal margin (Fig.4.15B).

41 -- 47 days (n=11, Table 4.2, 4.4)

Dramatic progress in the development of the retinal projection was demonstrated at this stage. A terminal arborization formed by the axons from the temporal retina but not from other quadrants, was first recognized in the retinotopically appropriate region.

Temporal deposit (n=5) Ganglion cell axons in five animals were labelled by DiI in the temporal retina. In three of them, the terminal branching was seen in the form of a localized band at the rostral border of the contralateral SC elongated along the medial-lateral collicular axis (Fig.4.17A). Within this particular region, which was termed a terminal zone, there was fine branching of axons crossing in different directions, mainly mediolaterally (Fig.4.18). The terminal zones covered regions with an average of 0.39 mm^2 of the collicular area (n=3). Still, many axons were

followed past the terminal zone. They extended caudally and were confined to the rostral two thirds of the SC. The axons covered predominantly the more medial side of the SC due to the tracer in the temporal retina being slightly ventral. The area covered by the temporal axons reached a peak with an average of 38% of the SC. From this stage, growth cones were not seen in large numbers and they generally had a simple morphology, lacking filopodia and lamellopodia. Now, the growth cones were only slightly larger than the parent axon. They were slender and rod-like (Fig.4.19). Ipsilaterally, there was an approximate mirror image to that seen on the other side. A terminal zone with fine branching and less dense than that seen contralaterally was formed in a mediolaterally orientated band at the rostral edge of the SC. Many axons were traced caudally past the zone, confined to the rostral two thirds of the SC (Fig.4.17A; Fig.4.20).

Nasal deposit (n=2) Labelled axons from the nasal retina spread rostrocaudally in the contralateral SC. The coverage of the nasal axons reached a peak with an average of 65% of the collicular area. As axons reached the caudal pole of the SC, they converged in a region which was the future site of the terminal zone, although no terminal branching was present yet. A couple of axons with simple growth cones were observed (Fig.4.17B). Axons side branches were rarely seen. No labelled axons or terminal zones were detected in the ipsilateral SC (Fig.4.17B).

Dorsal deposit (n=2) Labelled axons from the dorsal retinal deposit at this time were found to be positioned in the lateral contralateral SC, running rostrocaudally. The area covered by the axons in the SC was 16%. This distribution of labelling is analogous to that seen at earlier stages. No terminal arborization was observed in the labelled area (Fig.4.21A).

Ipsilaterally, labelled axons were not detected from the dorsal deposit in the retina.

Ventral deposit (n=2) Many labelled axons coming from a focal placement of DiI in the ventral retina dispersed rostrocaudally along the medial border of the contralateral SC, extending over an area of 19% of the SC. More axons were concentrated on the medial edge. Axons terminating in growth cones and axon branching were uncommon. No terminal zone could be detected (Fig.4.21B). Fewer labelled axons were organized as an approximate mirror image in the ipsilateral SC, where the axons concentrated along the medial border. A terminal zone was not present (Fig.4.21B).

52 -- 55 days (n=7, Table 4.2, 4.4)

From this stage, axons from all retinal quadrants began to arborize in their retinotopically appropriate region of the SC. The terminal zones were larger than mature terminals and occupied an area with an average of 0.30 mm^2 of the SC. There were also many axons still distributed outside the terminal zones in the contralateral SC.

Temporal deposit (n=2) By this age, more mature terminal zones with more highly branched axons and intense fluorescent label were formed by axons from the temporal retina in the rostral contralateral SC. As the deposit was slightly dorsal, axons were present in the more lateral SC. The terminal zone was characterized by a cloudy background filled with fine beaded branches. A denser region with more branching was found in the centre of this region. The edge of the terminal zone was not sharp (Fig.4.22A). Many labelled axons extended caudally past the terminal zone,

but they were confined to the rostralateral region of the SC (Fig.4.23A). The area covered by the axons in the contralateral SC was 14% on average. Axons with simple growth cones characterized as slender and rod-like were seen occasionally (Fig.4. 19). Ipsilaterally, a mirror image to that seen on the other side was present. A terminal zone was positioned rostrally close to the lateral border. Within the region, less axon branching, compared with that seen contralaterally, was observed (Fig.4.20). Many axons extended caudally past the terminal zone. They were confined to the rostrolateral part of the SC (Fig.4.23A).

Nasal deposit (n=3) The terminal zones formed by the nasal ganglion cell axons were first detected at the retinotopically correct caudal pole of the contralateral SC at this stage. Fine, beaded branches were distributed throughout the terminal region (Fig.4.22B) but they were much less intensely fluorescent than branches in the terminal zone from temporal axons and appeared less mature. Many axons were traced from the rostral SC to the terminal zone, with a couple of axons passing further caudal to it (Fig.4.23B). As well as the more widely distributed axons seen in roughly retinotopically correct regions at this and earlier ages, a more extreme example of overshooting of axons was seen at this age. Axons labelled by DiI in nasal and slightly ventral retina, extended rostrocaudally along the medial border of the SC and the terminal zone was formed in the mediocaudal SC, in the retinotopically correct region. Axons continued past the terminal zone to the caudal pole. At the caudal pole, some of these axons turned around to travel rostrally along the lateral edge of the SC. A few axons reached the rostral pole of the SC laterally (Fig.4.24). At this stage, the widely distributed axons covered an area of 39% of the SC.

The amount of branching of labelled nasal axons in the three animals was quantified to compare with the result at 27-28 days. This counting was made in the rostral and caudal half of the SC, respectively. At this stage, 12.6% (mean, SD=±2.1) of labelled axons had one small side branch (<=20 μm in length) in the rostral half of the SC, while 9.3% (mean, SD=±3.3) of labelled axons had a short side branch in the caudal half of the SC (Fig.4.25).

Neither terminal zones nor labelled axons from the nasal retina were seen ipsilaterally.

Dorsal deposit (n=1) A more mature terminal zone from the dorsal retinal axons was first detected in the retinotopically correct position in the lateral contralateral SC from this age. More branches and more intense fluorescent label could be clearly observed within this region (Fig.4.22C). Many axons approached the terminal zone from the rostral pole and extended caudally. The axons were confined to a band along the lateral edge of the SC, covering 20% of collicular area. One axon with a side branch was seen outside the main region (Fig.26A, 4.27). In the ipsilateral SC, a very few axons were observed along the lateral border (Fig.4.26A).

Ventral deposit (n=1) A terminal zone from ventral retinal ganglion cells was first detected at the medial border of the contralateral SC, in the retinotopically appropriate position. Within this immature terminal region (Fig.4.22D), compared with those formed by the temporal and dorsal retinal quadrants at this age, there was less axon branching and less intense fluorescent label. Axons were widely distributed outside the terminal zone and extended caudally along the medial part of the SC, covering 34% of the SC. More axons were concentrated on the most medial

side (Fig.4.26B). In the ipsilateral SC, a few axons extended along the medial border but did not reach the far caudal pole. A terminal zone was not seen (Fig.4.26B).

61 -- 68 days (n=12, Table 4.2, 4.4)

From this age, many of the initially more widely distributed axons labelled by DiI from all regions of retina had disappeared. Clearly defined terminal zones from all retinal quadrants were formed. Terminal zones occupied a mean area of 0.29 mm^2 in the collicular area. The organization of the retinal projection in the SC was close to maturity.

Temporal deposit (n=1) A clearly defined terminal zone formed by the ganglion cell axons from the temporal retina was present in the rostral contralateral SC. Distribution of the labelled axons outside the terminal zones was greatly reduced, to 3% of collicular area. A very few axons were distributed adjacent to this terminal region (Fig.4.28A). Within the terminal zone, the branching was extremely fine and large diameter branches were rare (Fig.4.29A). No labelling was seen in the ipsilateral SC.

Nasal deposit (n=6) Observations were made on six animals with DiI deposits in the nasal retina. In most of them, no axons could be followed from the rostral pole to the terminal zones which were confined to small regions in the caudal contralateral SC (Fig.4.28B), but a few axons were seen adjacent to the terminal zone (Fig.4.29B). Labelling on the ipsilateral SC was not detected in any of the animals with nasal deposits of DiI.

Dorsal deposit (n=3) A terminal zone was found in the lateral contralateral SC. A few axons were traced from the rostral pole to the zone

and a couple of axons could be detected caudally to it (Fig.4.30A). The terminal zone was clearly defined with a relatively sharp edge, compared with that seen at earlier stages. Fine branching of axons was distributed within this region (Fig.4.29CD). Terminal zones or labelled axons were not seen ipsilaterally.

Ventral deposit (n=2) After a DiI deposit in the ventral, slightly temporal retina, a defined terminal zone was found to be positioned medially in the contralateral SC, with some axons coming to it from the rostral pole. A few axons extended further caudally along the lateral border of the SC (Fig.4.30B). One axon tipped with a growth cone was labelled in the medial ipsilateral SC but no terminal zone was seen (Fig.4.30B).

Quantification of DiI deposits in the retina and labelled axons and terminal zones in the SC

The size of the DiI deposit in the retina ranged on average from 0.8% to 5.2% of retinal area at different age groups (Fig.4.31C). Small differences in the area of DiI deposit in the retina were not found to correlate with the area covered by labelled axons in the contralateral SC (Fig.4.31A). However, the collicular area covered by labelled axons and by terminal zones correlated with age (Fig.4.31A, B, D)

Retinotopically, axons from each quadrant of retina only covered part of the SC. From the early developmental stage, the distribution of labelled axons in the contralateral SC increased gradually with ages. Subsequently, from 41 to 55 days when retinal axons from all quadrants formed terminal zones, the area covered by the temporal or nasal axons reached a mean peak coverage of around 35% and 65% of the SC, respectively. Similarly,

axons from ventral and dorsal retina reached a mean peak coverage of 20-34% of the SC. Thereafter, the area of the SC covered by labelled axons reduced with time (Fig.4.31A).

Both the absolute size and the percentage of the area covered by terminal zones in the SC formed from all retinal quadrants decreased on average from 41-47 days when they were first detected, to 90-95 days when the mature discrete ones were present (Fig.4.31B). In addition, the area of terminal zones formed from each retinal quadrant is also shown. These also declined with development (Fig.4.31D; Table 4.7-9).

There were 11.6% of nasal axons forming side branches in either the rostral or the caudal half of the SC prior to appearance of terminal zones at 27-28 days. A similar number of branches was seen at the time of formation of terminal zones at 52-55 days, in which there were 9.3% and 12.6% of nasal axons with side branches in the rostral and caudal SC, respectively (Fig.4.32)

Table 4.1 Percentage of retina covered by the DiI deposit and percentage of the SC covered by labelled axons at 12-13 to 90-95 days

Ages (days)	*Retinal quadrant*	*% of retina covered by DiI deposits*	*% of SC covered by axons*
90-95	T	1.6	-
		0.9	-
		3.4*	2
	N	0.8	-
		0.8	40
	D	1.3*	6
	V	2.3	-
		4.7	-
	Mean	1.9	
	Mean*	2.4	
12-13	T	8.1	16
	N	5.9	20
	D	0.9	18
	V	5.7	18
	Mean	5.2	
22-24	T	2.9	14
		6.7*	22*
	N	3.5	24
	D	1.0	18
		0.4	7
	V	3.4	14
	Mean	2.2	
27-28	T	1.4	14
		3.5	22
		2.5*	22*
	N	1.0	34
		1.8	26
		6.7	38
		2.9	94
		4.6*	54*
	D	1.5	11
		2.4	15
	V	1.1	20
		1.7	36
		2.2*	38*
	Mean	2.4	
	Mean*	3.1	

T: temporal, N: nasal, D: dorsal and V: ventral.
* indicates deposits of DiI in the more central retina.

Table 4.2 Percentage of retina covered by the DiI deposit and percentage of the SC covered by labelled axons at 41-47 to 61-68 days

Ages (days)	*Retinal quadrant*	*% of retina covered by DiI deposits*	*% of SC covered by axons*
41-47	T	2.3	15
		2.9	43
		0.9	53
		1.3	41
		1.1	40
	N	2.0	73
		1.6	57
	D	0.5	14
		4.2	17
	V	0.6	22
		0.8	15
	Mean	1.7	
52-55	T	1.4	6
		0.8	22
	N	2.9	43
		1.9	32
		3.2	41
	D	0.7	20
	V	1.5	34
	Mean	1.8	
61-68	T	1.0	3
	N	2.1	-
		0.9	-
		1.2	-
		0.8	-
		0.1	1
		0.8	40
	D	1.3	3
		0.6	4
		0.8	12
	V	0.2	8
		0.8	38
	Mean	0.9	

T: temporal, N: nasal, D: dorsal and V: ventral.

Table 4.3 Area of terminal zones and percentage of the SC covered by terminal zones at 90-95 days

Ages (days)	*Retinal quadrants*	*Area of SC covered by terminal zones (mm^2)*	*% of SC covered by terminal zones*
90-95	T	0.04	0.2
		0.05	0.3
		0.08	0.4
	Mean	0.06	0.3
	N	0.20	0.8
		0.06	0.3
	Mean	0.13	0.6
	D	0.14	0.7
	V	0.10	0.6
		0.13	1.0
	Mean	0.12	0.8

T: temporal, N: nasal, D: dorsal and V: ventral.

Table 4.4 Area of terminal zones and percentage of the SC covered by terminal zones at 41-47and 61-68 days

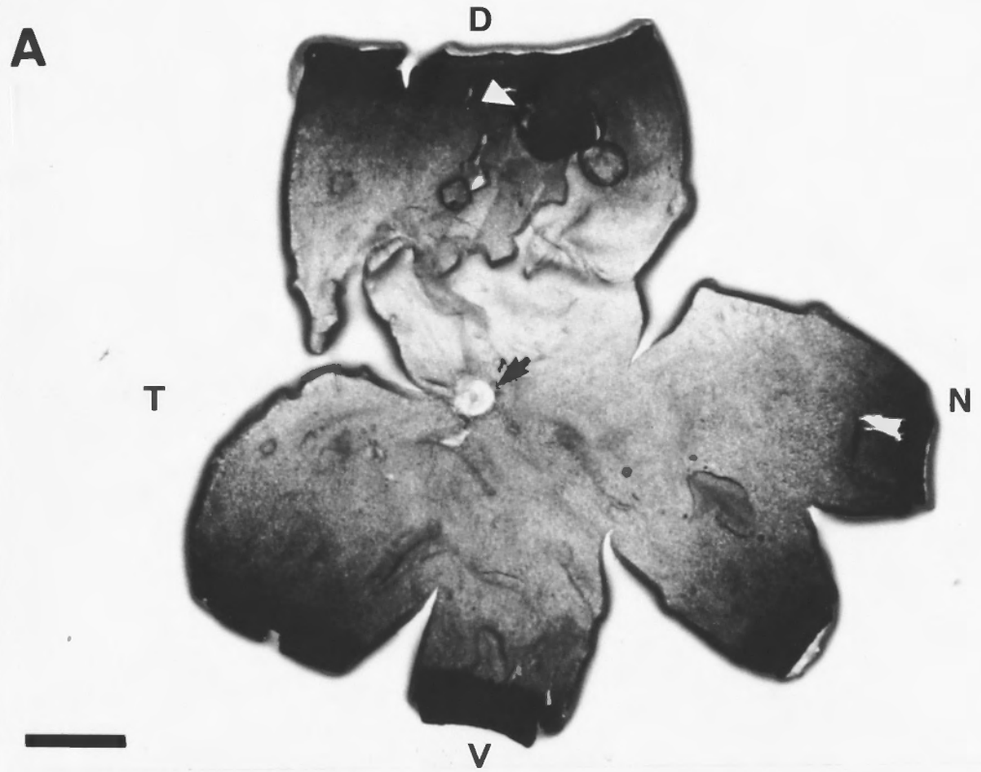
Ages (days)	*Retinal quadrant*	*Area of SC covered by terminal zones(mm^2)*	*% of SC covered by terminal zones*
41-47	T	0.36	2.6
		0.45	5.4
		0.36	3.3
	Mean	0.39	3.8
52-55	T	0.27	1.5
		0.24	2.4
	Mean	0.26	2.0
	N	0.52	3.7
		0.20	1.3
		0.20	2.4
	Mean	0.31	2.5
	D	0.24	1.6
	V	0.43	3.4
61-68	T	0.16	0.9
	N	0.09	0.5
		0.04	0.5
		0.55	4.3
		0.67	1.2
		0.15	0.8
		0.42	4.0
	Mean	0.32	1.9
	D	0.15	0.9
		0.22	1.7
		0.56	2.5
	Mean	0.31	1.7
	V	0.16	0.9
		0.31	2.4
	Mean	0.24	1.7

T: temporal, N: nasal, D: dorsal and V: ventral.

Figure 4.1 Photomicrographs of wholemount of a retina showing the position of the deposit of DiI and labelled axons at 28 days

(A): Position of DiI deposit. Bright-field view of a wholemount of an unstained retina shows the optic disc and the DiI deposit. The optic disc is indicated by an arrow. The DiI deposit (arrow head) is positioned in the periphery of the dorsal retina. T: temporal; N: nasal; D: dorsal; V: ventral. Bar: 1 mm.

(B): Fluorescent Image of DiI Labelling in A. An arrow indicates the focal DiI deposit in the periphery of the dorsal retina. Labelled axons form a compact bundle and they are traced from the deposit site to the optic disc (OD). As the labelled axons enter the OD, they gather together and exit the eye. The light area (arrow head) beside the labelled axons is not fluorescent labelling but is caused by a fold in the tissue as can be seen in the bright field view in A. Bar: 1 mm.


A
D
T
N
V

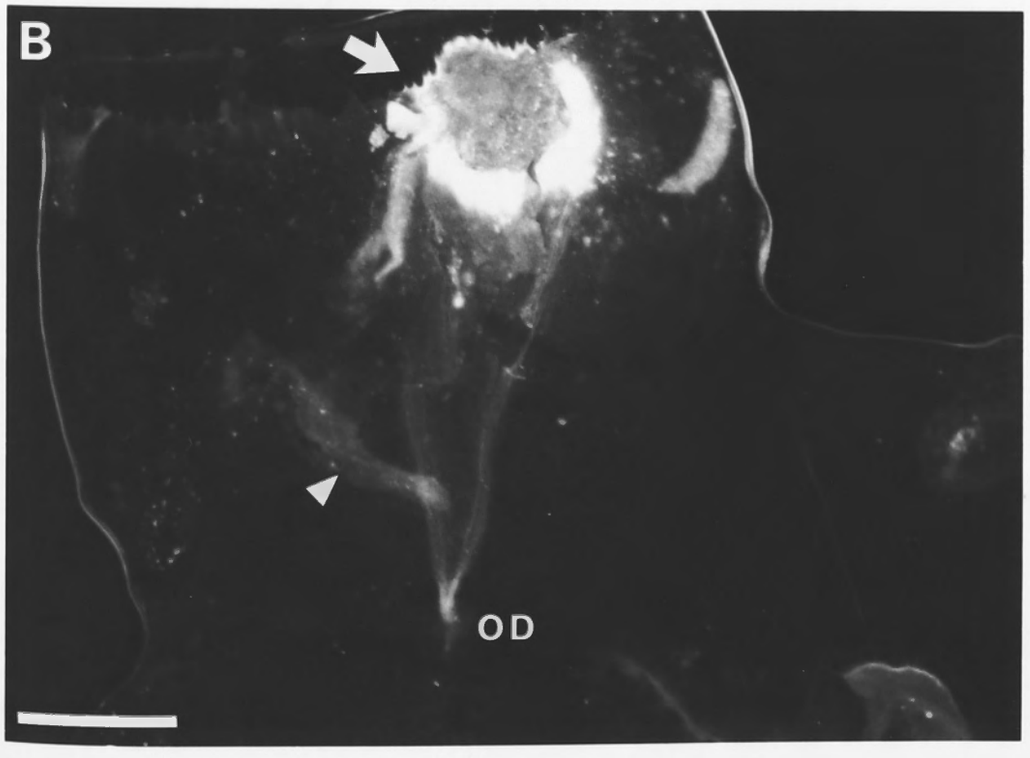

B
OD

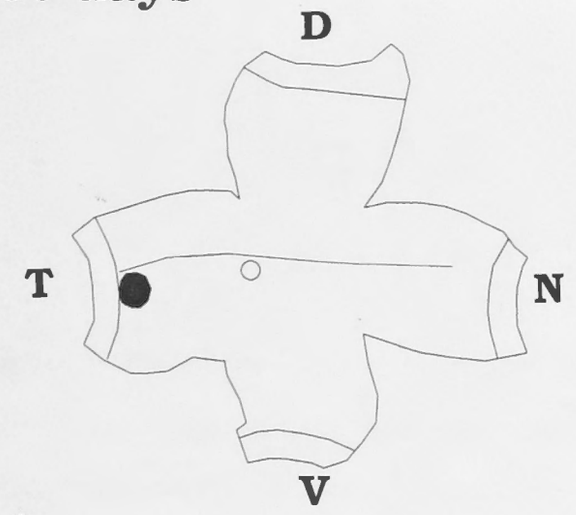
Figure 4.2 Camera lucida drawings of wholemounts of the retina and the SC after deposits of DiI in the temporal and nasal retina at 90-95 days

Top: Location of deposits of DiI in the retina. DiI deposits in each quadrant of retina are in solid black. Open circle marks the optic disc (OD). The line crossing temporonasally is the pigment line which indicates the border of light (upper) and heavy (lower) pigmentation. Labelled axons can be seen between the deposit and the OD in B. The labelled region is outlined rather than every individual axon being drawn. T: temporal; N: nasal; D: dorsal; V: ventral. Bar: 1mm.

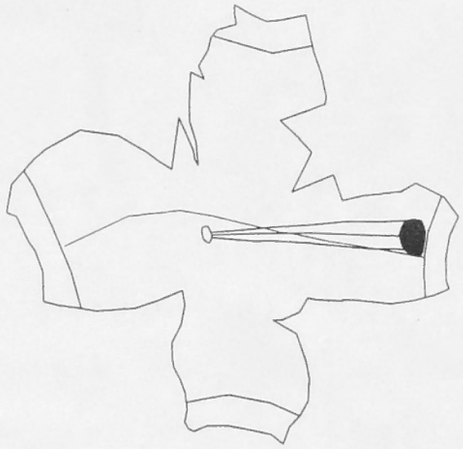
Bottom: Position of the retinal ganglion cell terminals in the SC. Right hand side is the SC contralateral to the eye with DiI deposit. Outline of the SC is shown. The terminal zones in the SC are in solid black. R: rostral; C: caudal; M: medial; L: lateral; Contra: contralateral; Ipsi: ipsilateral. Bar: 1 mm.

Discrete terminals are localized in the rostral and the caudal SC, respectively, after DiI deposits in the periphery of the temporal (A) and nasal retina (B).

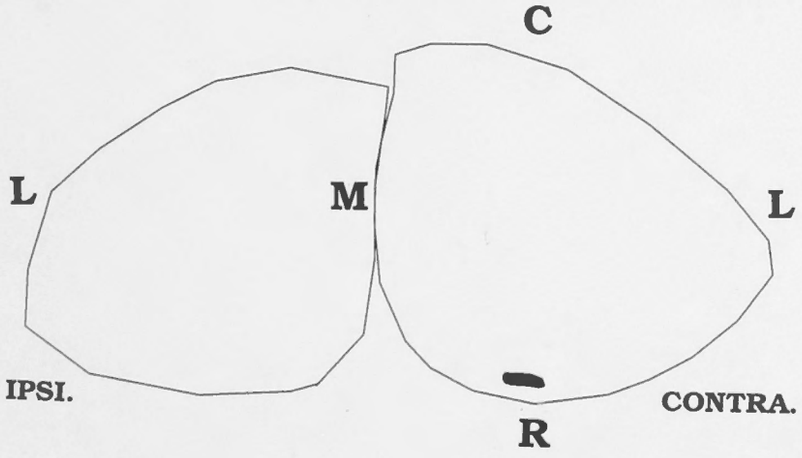
90-95 days



A



B



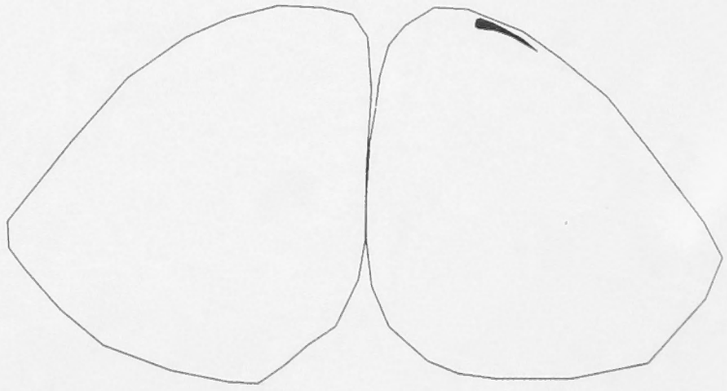
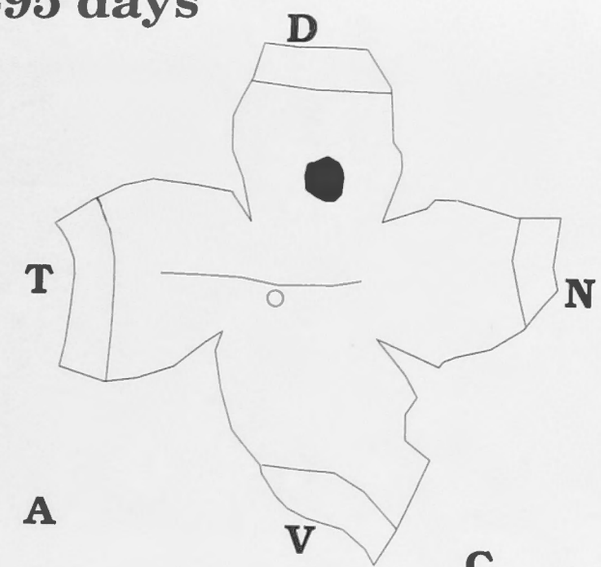
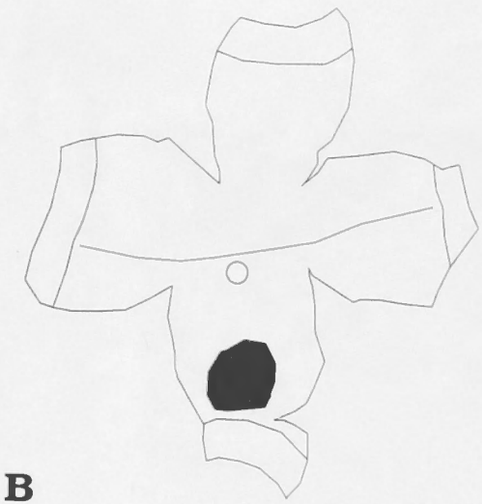


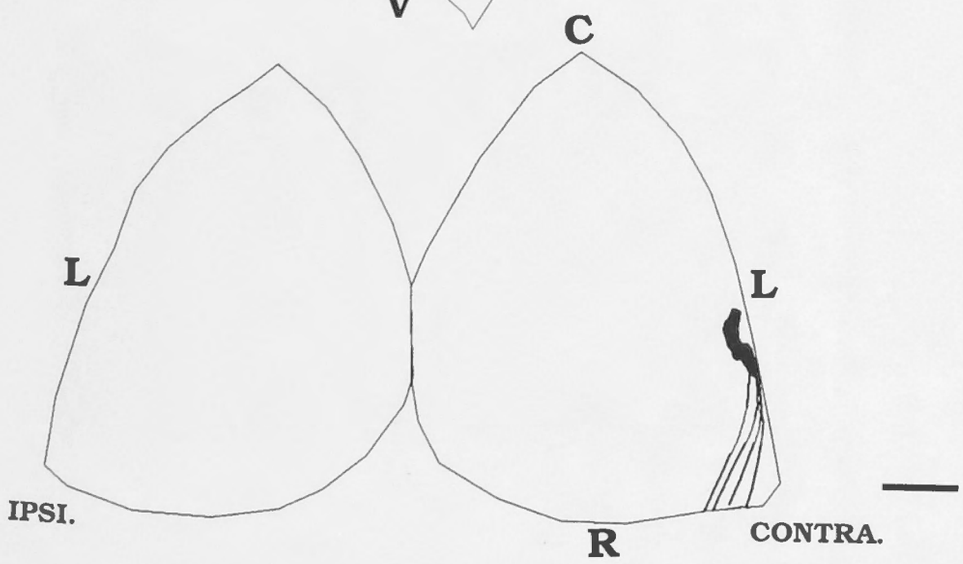
Figure 4.3 Camera lucida drawings of wholemounts of the retina and the SC with dorsal and ventral deposits of DiI at 90-95 days

Conventions are the same as for figure 4.2. **(A)**: With labelling from more central dorsal retina, a discrete terminal zone is positioned laterally in the contralateral SC. A few axons run from the rostral pole to the zone. **(B)**: A focal terminal zone is localized in the medial contralateral SC after a large dye deposit in the ventral retina. Bars: 1 mm.

90-95 days







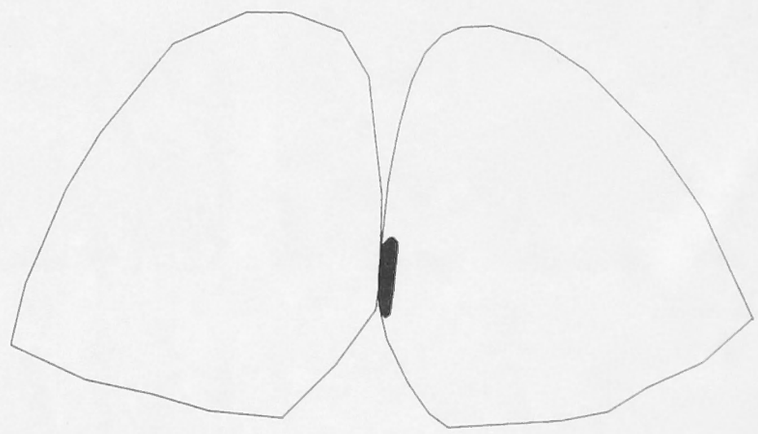
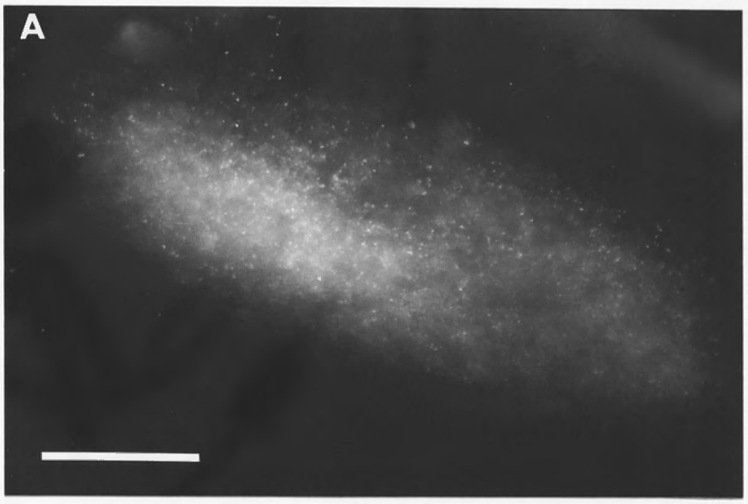
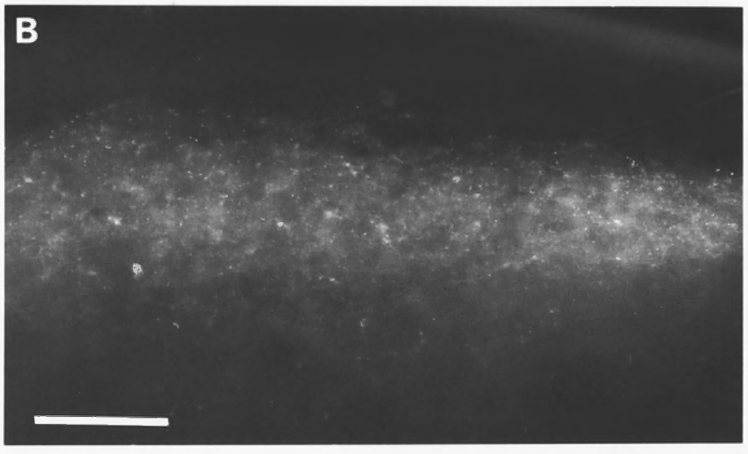
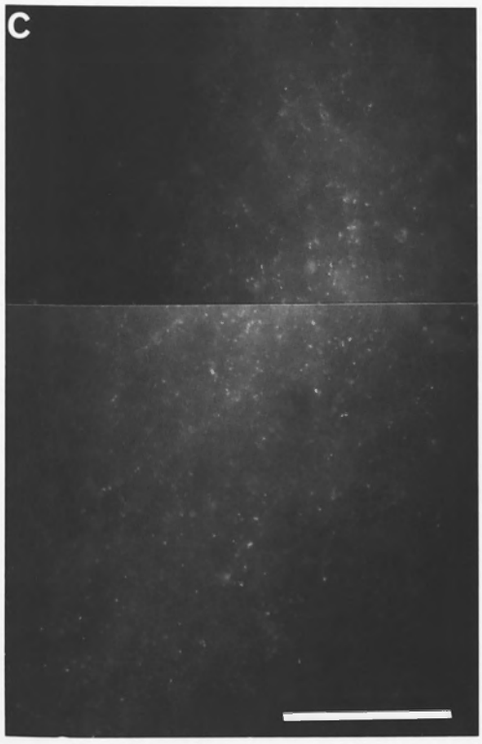


Figure 4.4 Fluorescent images of terminal zones in the contralateral SC with DiI deposits in the temporal, nasal, dorsal and ventral retina at 90-95 days

(A): A discrete terminal zone is present in the rostral SC after a temporal deposit in the retina. Fine spots fill this region on a cloudy background. **(B):** A focal terminal zone is shown in the caudal SC, after a nasal deposit of DiI in the retina. **(C)** shows a discrete terminal zone from dorsal axons. It also is filled with fine spots on a cloudy background. **(D):** An image from a sagittal section demonstrates a focal terminal zone after a deposit of DiI in the ventral retina. Fine branching can be seen within the zone in this sectioned material. Axons distributed outside the region are not seen. Bars: 100 μm.


A


B


C

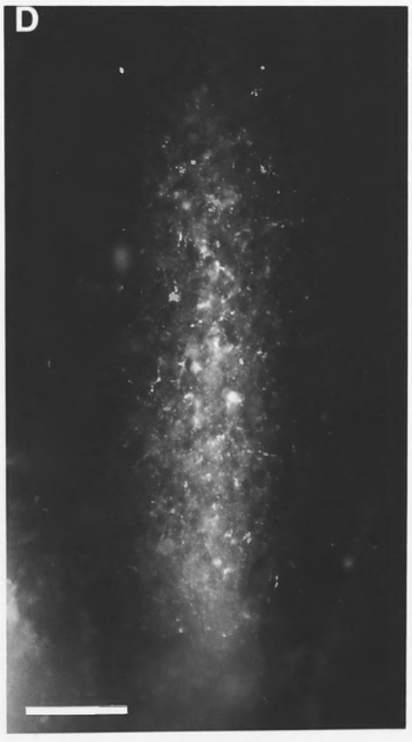

D

Figure 4.5 Camera lucida drawings of wholemounts of the retina and the SC at 12-13 days

Top: Location of DiI deposits in the retina. DiI deposits in each of four quadrants in the temporal, nasal, dorsal and ventral retina are in solid black. Open circle marks the optic disc (OD). Labelled axons are followed between deposits and the OD. Not every labelled axon is drawn but the labelled areas are outlined. T: temporal; N: nasal; D: dorsal; V: ventral. Bar: 1 mm.

Bottom: Distribution of the ganglion cell axons in the SC. Sizes of the SC vary slightly at similar ages because of flattening to produce the wholemounts. Right hand side is the SC contralateral to the eye with DiI deposits. Outline of the wholemounts and labelled axons are drawn (see methods). Small filled spots show axons ending in a growth cone. R: rostral; C: caudal; L: lateral. Contra: contralateral; Ipsi: ipsilateral. Bar: 1 mm.

(A): Labelled axons arising from a DiI deposit in the temporal retina extend into the contralateral SC and are confined to the rostral half. In mediolateral extent, axons are distributed in approximately one third of the SC with no axons in the most lateral or medial region. All labelled axons follow a rostrocaudal course through the SC and the adjacent axons tend to be aligned approximately in parallel. The fibres contribute evenly throughout the whole labelled region, with only two axons outside but close to the main region. Axons with growth cones are seen within the region where axons are distributed at all rostrocaudal levels. In the ipsilateral SC, a few temporal axons with growth cones are distributed at the rostral edge. **(B):** Many axons from the nasal retina extend across the mediolateral axis and reach the caudal half of the contralateral SC although they are yet to reach the far caudal pole. Labelled axons run primarily in a rostrocaudal direction and most of them are evenly distributed within a mediolaterally confined band. Only a couple of axons lie outside but adjacent to this region. Due to the slightly ventral deposit, the band is positioned close to the medial border, the target of ventral axons. Axons tipped with growth cones are spread throughout the labelled region. **(C):** Axons from the dorsal retina are distributed in the contralateral SC. They extend in the more lateral region of the rostral half of the SC and run in a primarily rostrocaudal direction. Axons with growth cones are scattered throughout the labelled area. Two axons tipped with growth cones are labelled at the lateral part of the ipsilateral SC. **(D):** Axons from the ventral retina enter the medial part of the contralateral SC and they are aligned rostrocaudally. No labelled axons are distributed in the lateral two thirds of the SC. The axons with growth cones are distributed throughout the labelled region. Note that branching is rare.

12-13 days

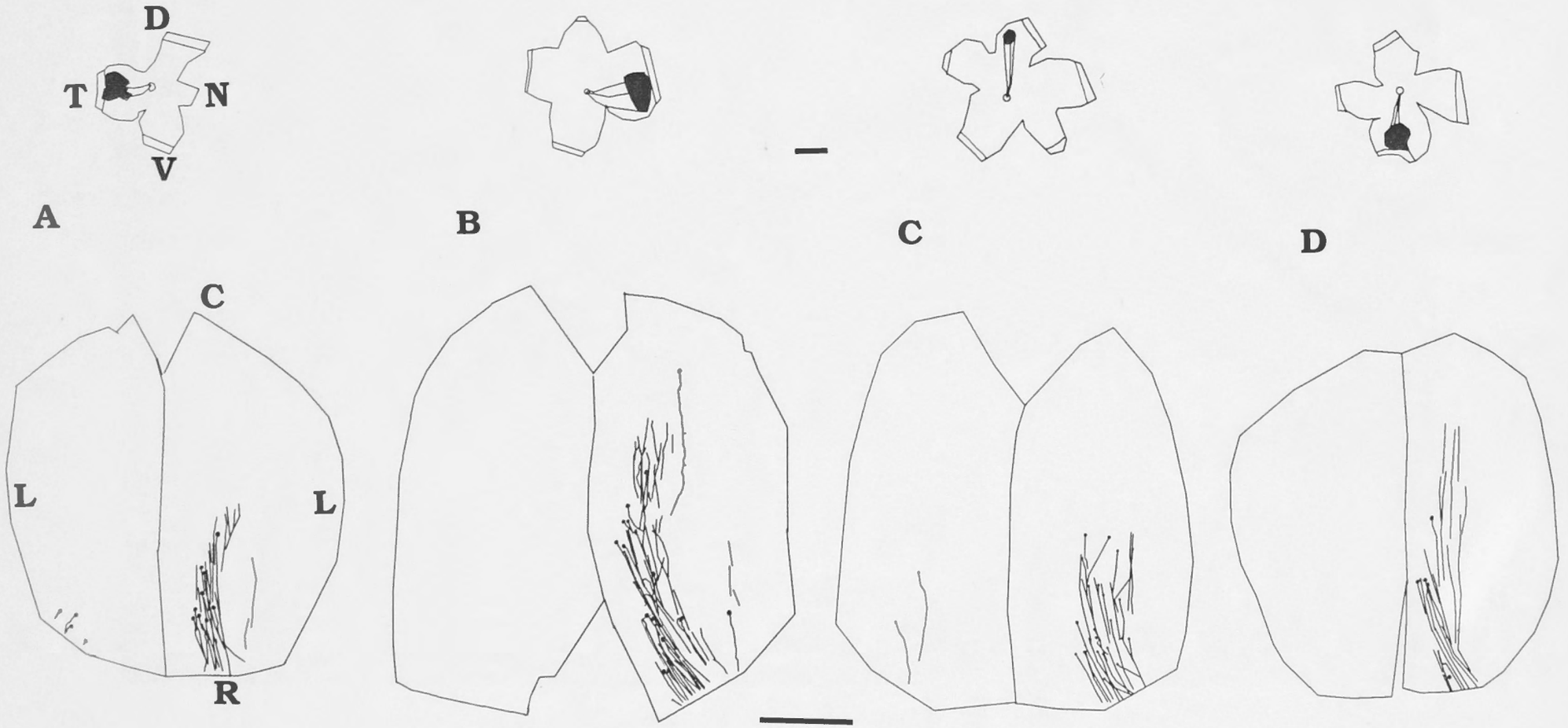
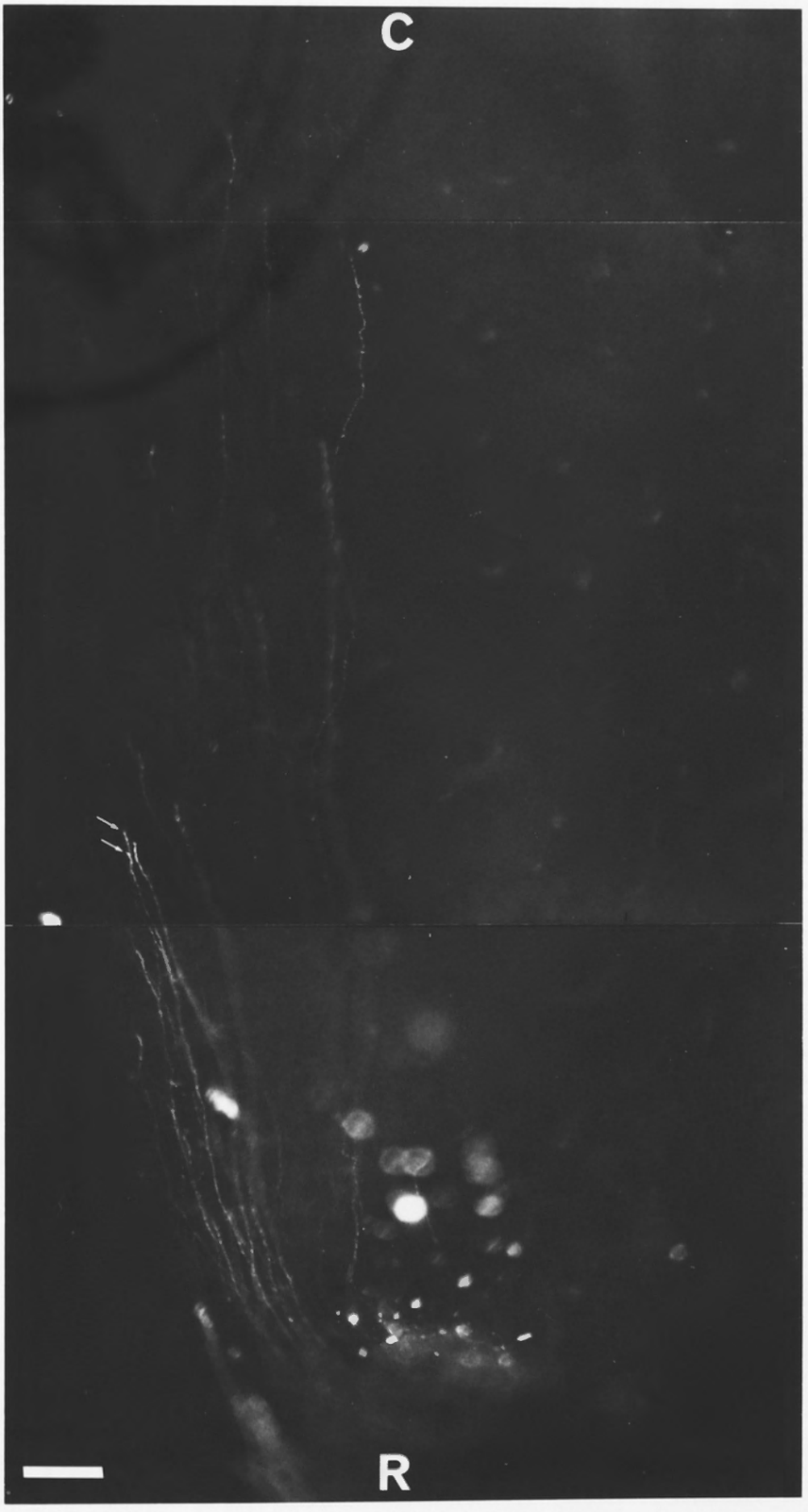


Figure 4.6 Photomontage of representative wholemount of the contralateral SC after a DiI deposit in the nasal retina at 13 days

Many labelled axons are followed primarily in a rostrocaudal direction and run straight to the caudal pole without branching. Arrows point out growth cones on the axons. Examples of these are shown in higher power in the following two figures. R: rostral; C: caudal. Bar: 100 μm.


C
R

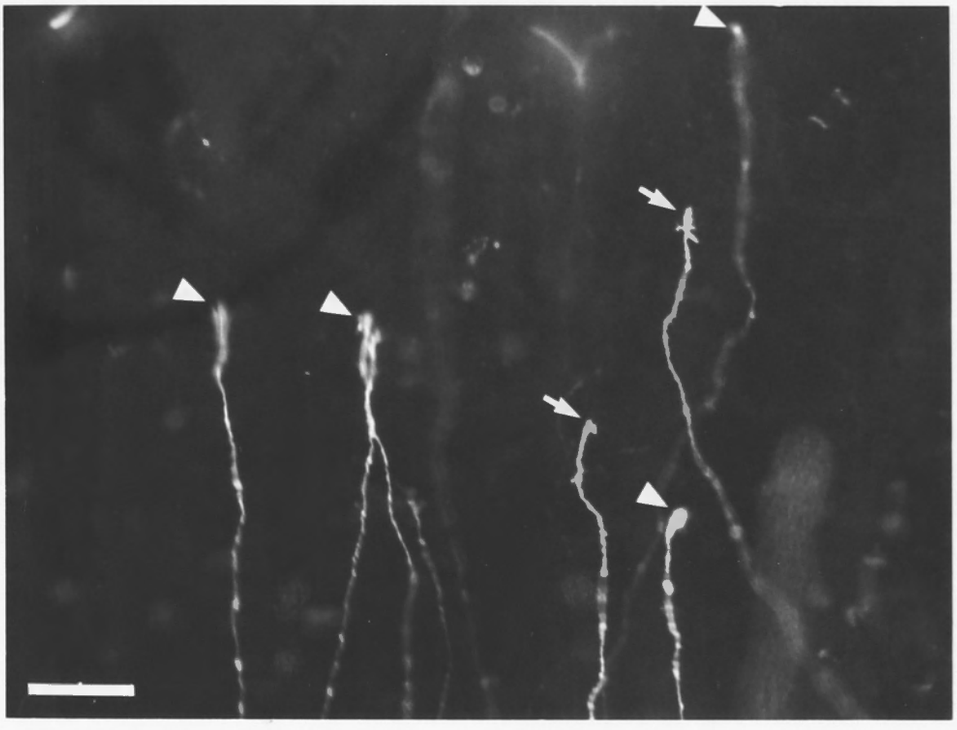


Figure 4.7 Fluorescent image of labelled axons ending in growth cones in the SC at 12 days

Arrows indicate growth cones at the tips of axons which run in a rostrocaudal direction. Arrow heads point to growth cones that are out of focus. Bar: 50 μm.

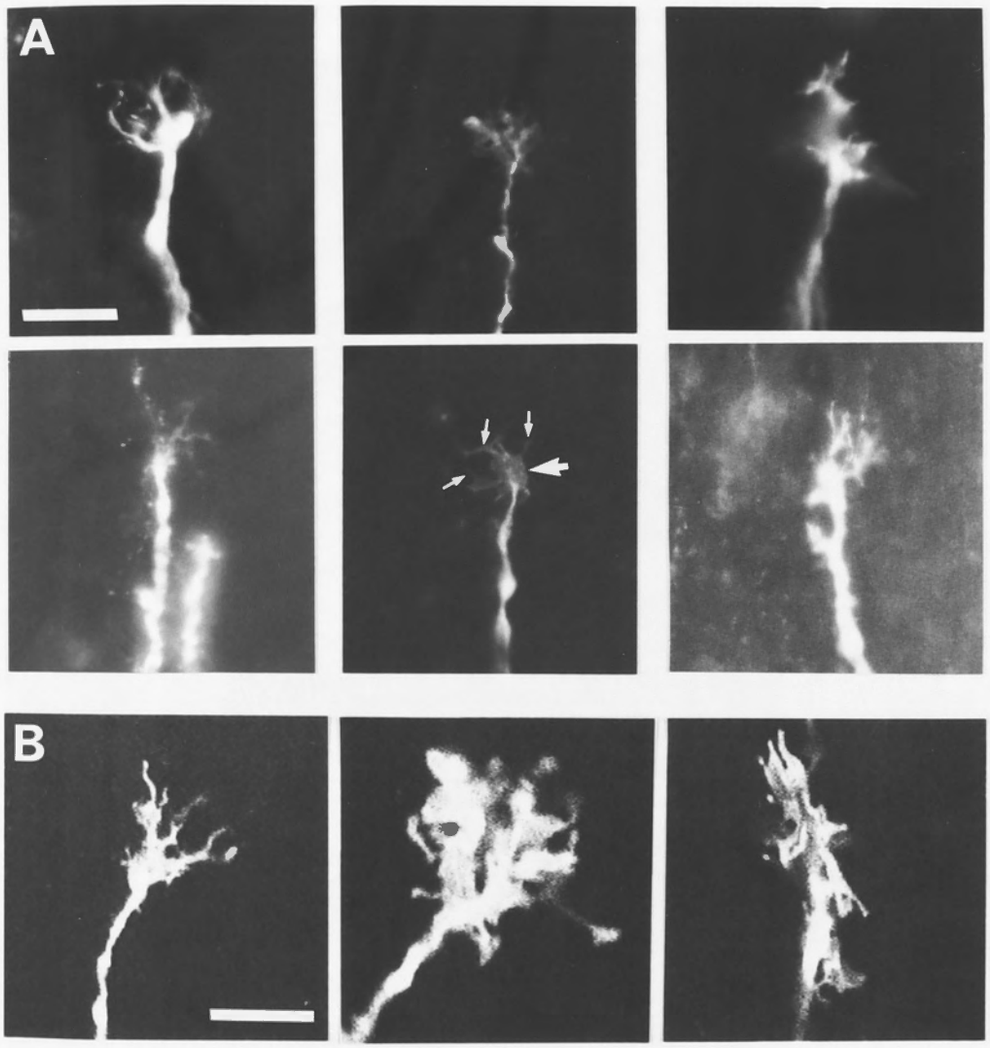


Figure 4.8 Fluorescent images of complex growth cones in the SC at 12 to 28 days

The morphology in this group of individual growth cones are defined as "complex". These complex growth cones consist of lamellopodia (large arrow) and filopodia (small arrow). Photomicrographs in A are taken on a fluorescence microscope and in B on a laser scanning confocal microscope. Bar: 20 μm.

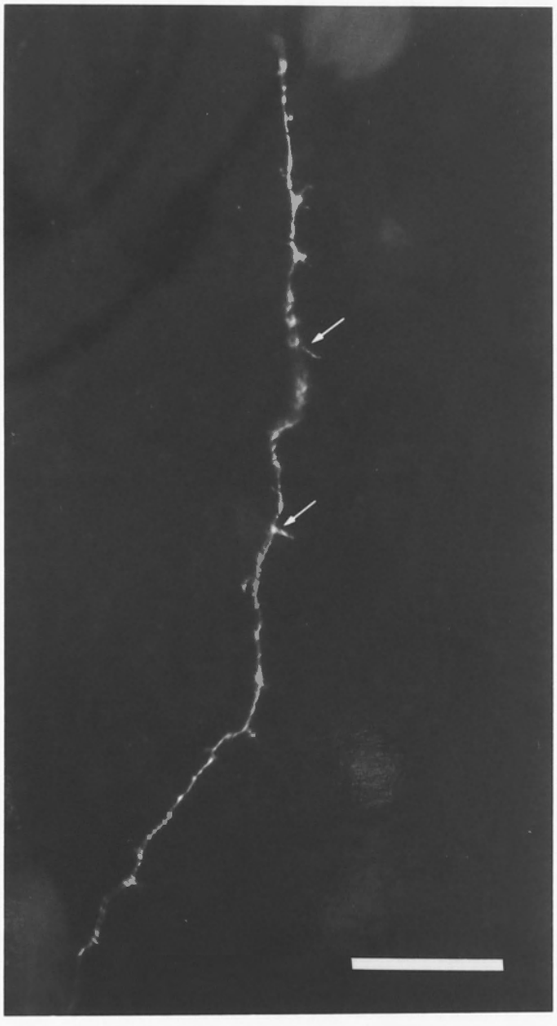


Figure 4.9 Fluorescent image of a rare labelled axon with small side branches at 12 days

Arrows point to small side branches. They are in the form of filopodia-like extensions along the parent trunk which runs rostrocaudally. Bar: 50 μm.

Figure 4.10 Camera lucida drawings of wholemounts of the retina and the SC showing the deposits of DiI in the retina (top) and the distribution of the labelled axons in the SC (bottom) at 22-24 days

Conventions are the same as for figure 4.5. **(A):** Labelled axons from a deposit of DiI in the temporal retina extend both contralaterally and ipsilaterally, confined to regions at the rostral SC. No axons are distributed outside the region. Many axons (not every one shown here) tipped with growth cones are spread evenly throughout the labelling. These projections on both sides of the SC are more or less a mirror image about the medial axis. **(B):** Labelled axons from the nasal retina extend toward the far caudal pole contralaterally along the medial border. Many axons ending in growth cones are seen in the labelled region. One labelled axon from the nasal retina is seen in the rostral ipsilateral SC. **(C):** Many axons from the dorsal retina are uniformly distributed in the lateral part of the contralateral SC with axons extending caudally. Growth cones are scattered throughout the labelled region. One axon from the dorsal retina with a growth cone enters the lateral ipsilateral SC. **(D):** Many ventral axons enter the contralateral SC at the rostral edge and extend caudally. The axons run along the more medial SC in a narrow band, with only one axon outside this region. The axons do not reach the medial most border of the SC. Labelled axons tipped with growth cones are distributed over the labelled region. A few ipsilateral axons from the ventral retina are traced medially in a rostrocaudal band, but they do not extend as far caudally as the contralateral ones. Bars: 1mm.

22-24 days

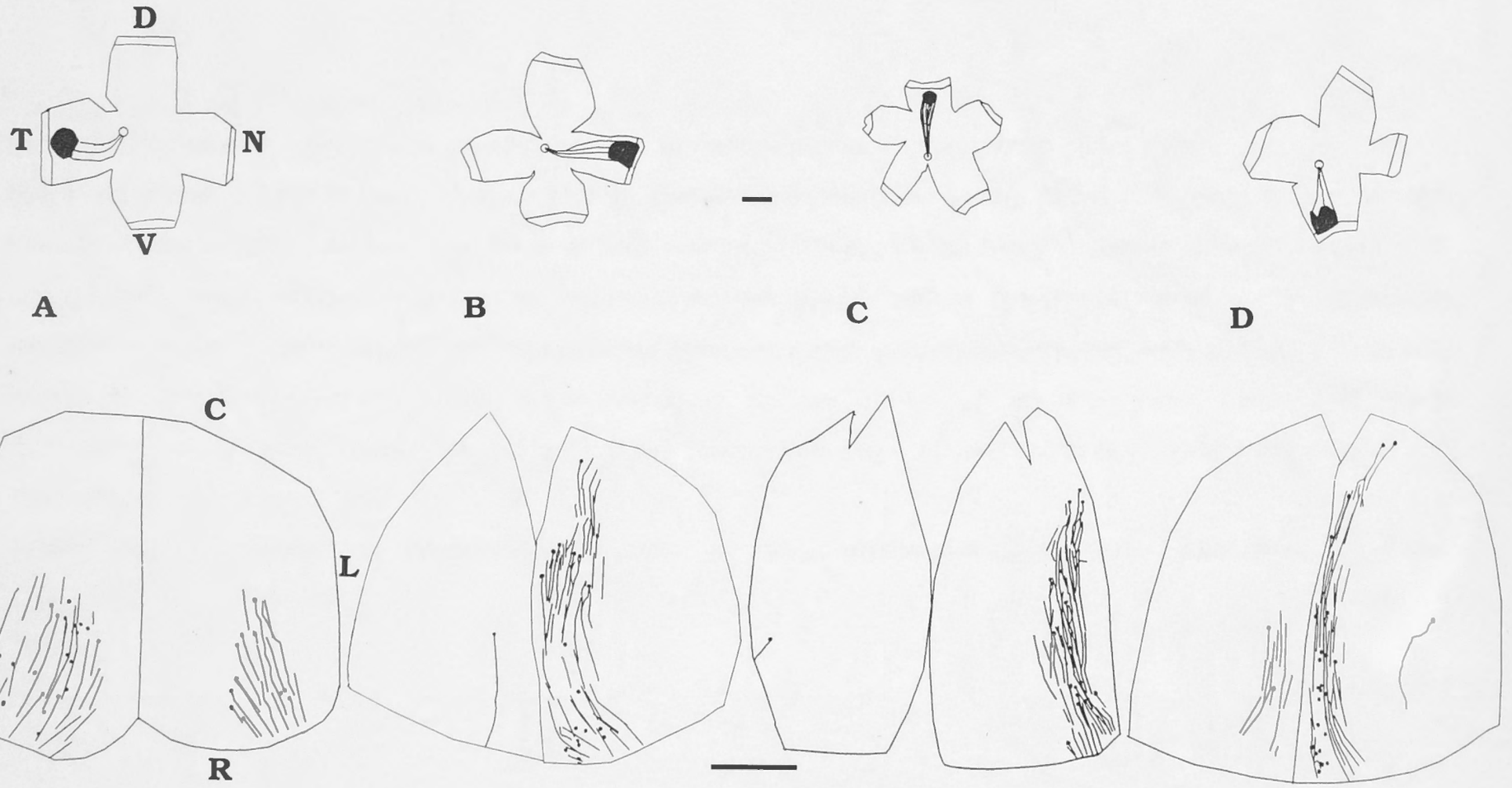
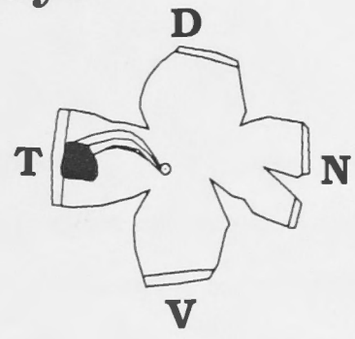


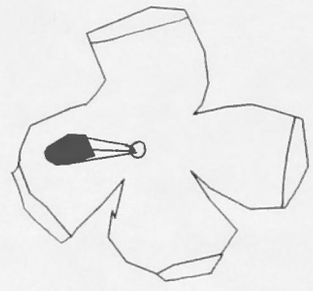
Figure 4.11 Camera lucida drawings of wholemounts of the retina and the SC with deposits of DiI in the temporal retina at 27-28 days

Conventions are the same as for figure 4.5. **(A):** A DiI deposit was placed in the periphery of the temporal retina and slightly dorsal. Axons project to the rostral SC contralaterally and ipsilaterally. The axons, without significant branching, run primarily in a rostrocaudal direction and they are evenly distributed within the confined regions in the both lateral SC. The axons ending in growth cones are spread throughout the labelled regions. **(B):** Axons from a more central deposit in temporal retina extend in the rostral parts on both contralateral and ipsilateral SC, similar to those derived from the peripheral retinal deposit of dye. There are slightly more axons distributed evenly in the contralateral side than the ipsilateral. Axons with growth cones are distributed in the labelled regions on both sides. Bars: 1 mm.

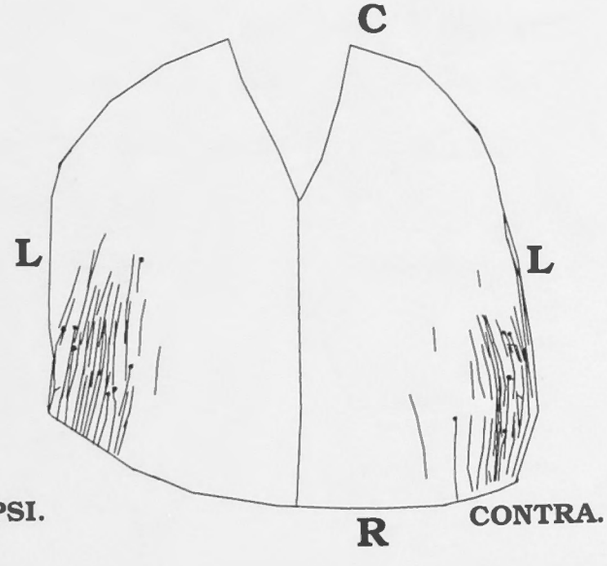
27-28 days



A



B



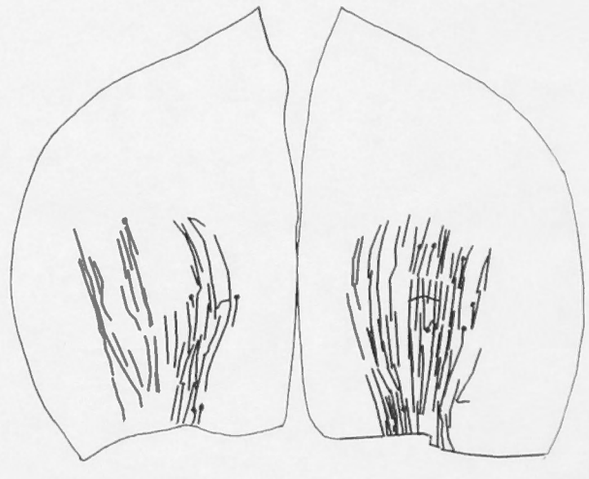


Figure 4.12 Camera lucida drawings of wholemounts of the retina and the SC with DiI deposits in the nasal retina at 27-28 days

Conventions are the same as for figure 4.5. This illustrates that small differences in placement in nasal retina change the course that nasal axons take to reach the caudal SC. **(A):** With a deposit in the nasal retina that extends slightly more into ventral rather than dorsal retina, labelled axons can be followed from the rostral pole to the caudal pole in the SC, and the axons are confined to a region close to the medial border and approximately one third of the mediolateral extent. Axons with growth cones are scattered in the labelled region. **(B):** Here, the deposit in the nasal retina extends into both dorsal and ventral retina and labelled axons in the retina are traced to the OD in two bundles, dorsally and ventrally. Consequently, the labelled axons in the SC run rostrocaudally across the whole mediolateral extent of the contralateral SC. Individual axons are not shown as they were so numerous. Instead, the positions of growth cones are shown. A micrograph illustrating the labelled axons is shown in the following figure. Bars: 1 mm.

27-28 days

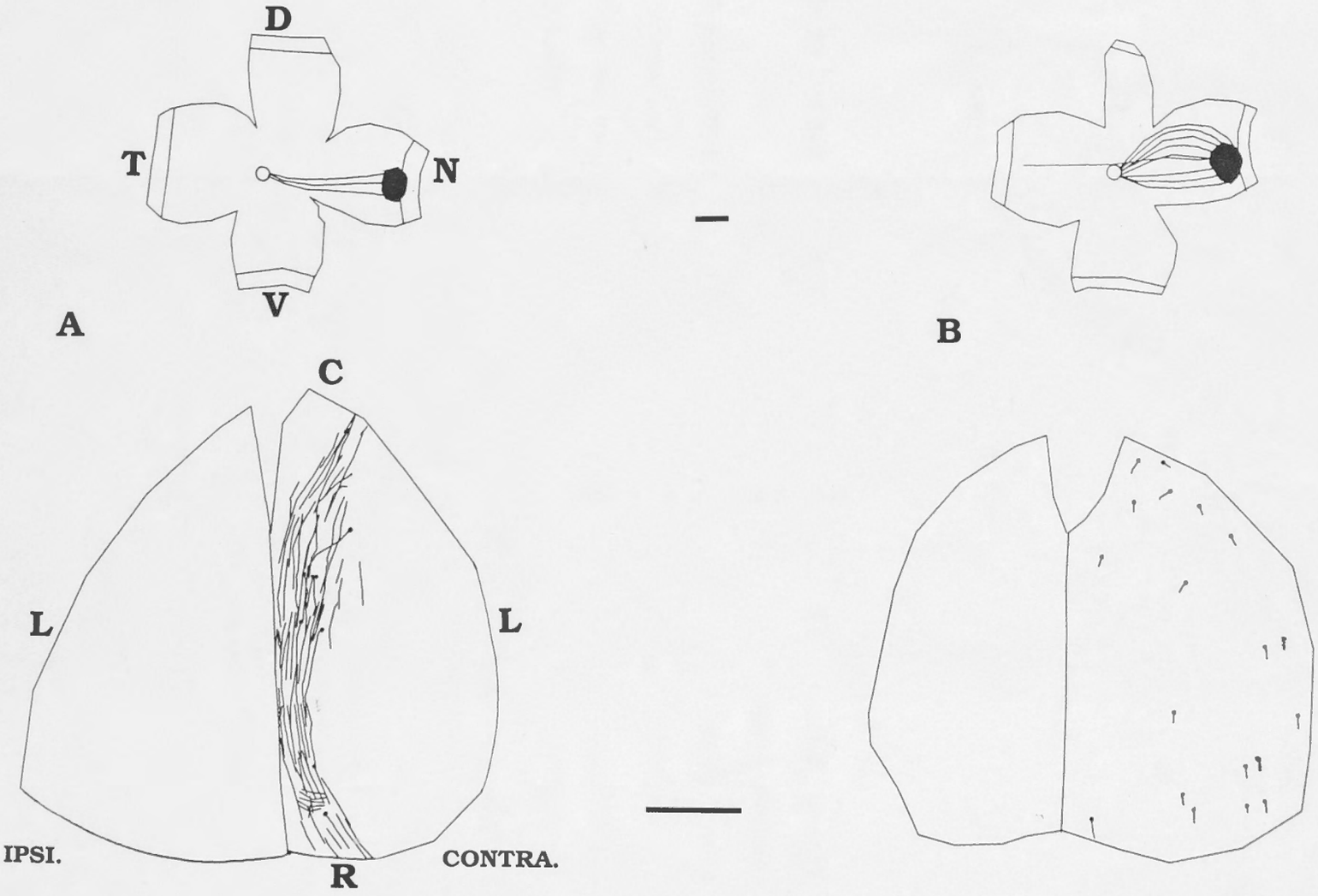


Figure 4.13 Fluorescent image of labelled axons in the contralateral SC at 28 days

A view of the lateral SC of the wholemount in figure 4.12B shows that many labelled axons extend rostrocaudally and the individual axons are aligned in parallel. Arrows point to the growth cones which are scattered at all levels of the rostrocaudal extent of the SC. There is little or no branching. R: rostral; C: caudal. Bar: 200 μm.


C
R
200 µm

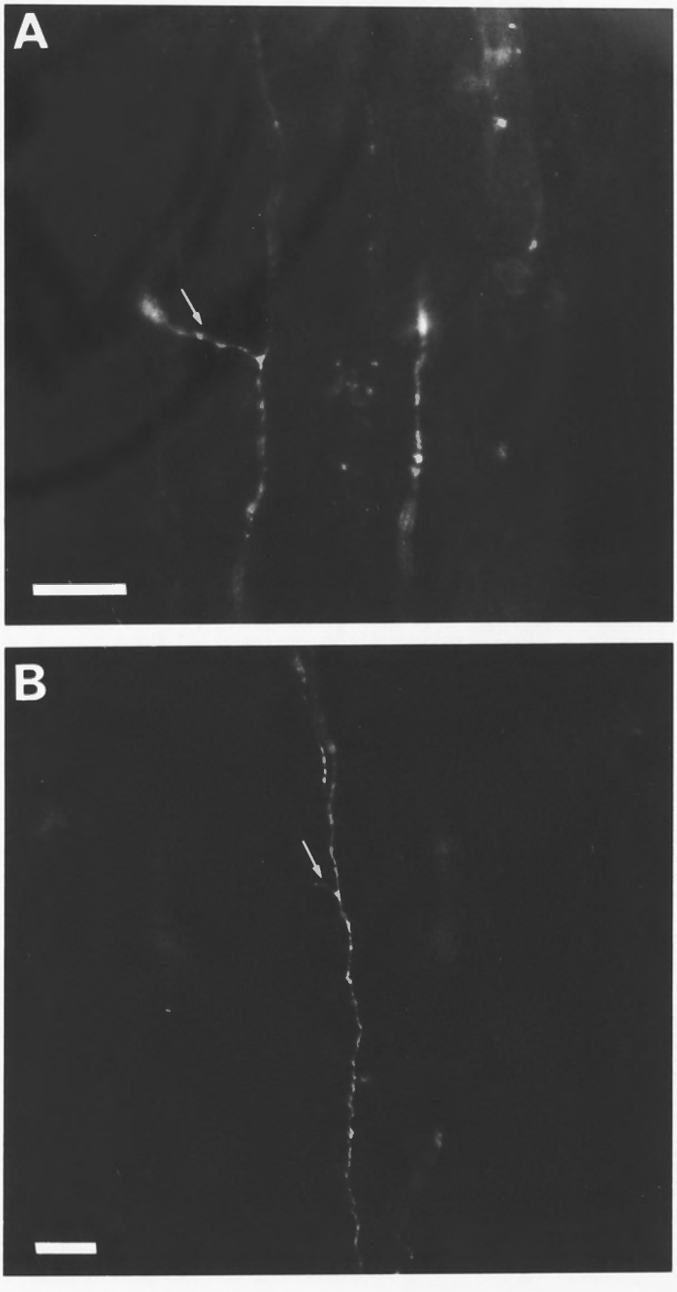


Figure 4.14 High power fluorescent image of branched labelled axons in the SC at 28 days

(A) and (B). Arrows point to small side branches derived from the parent axon. Other labelled axons run rostrocaudally without branching. Bar: 20 μm.

Figure 4.15 Camera lucida drawings of wholemounts of the retina and the SC with dorsal and ventral deposits at 27-28 days

Conventions are the same as for figure 4.5. **(A):** Axons from the dorsal retina are confined to a narrow band in the lateral SC. The axons are distributed evenly throughout the labelled region. Only one axon is labelled in the lateral ipsilateral SC. **(B):** Axons from the ventral retina are distributed medially in both the contralateral and ipsilateral SC. Contralaterally, axons extend rostrocaudally in a narrow band along the medial border of the SC and are concentrated mainly on the rostral two thirds of the SC. In the ipsilateral SC, axons are distributed similarly to those contralaterally, with the exception that no axons reach the far caudal pole. Bars: 1 mm.

27-28 days

A

B

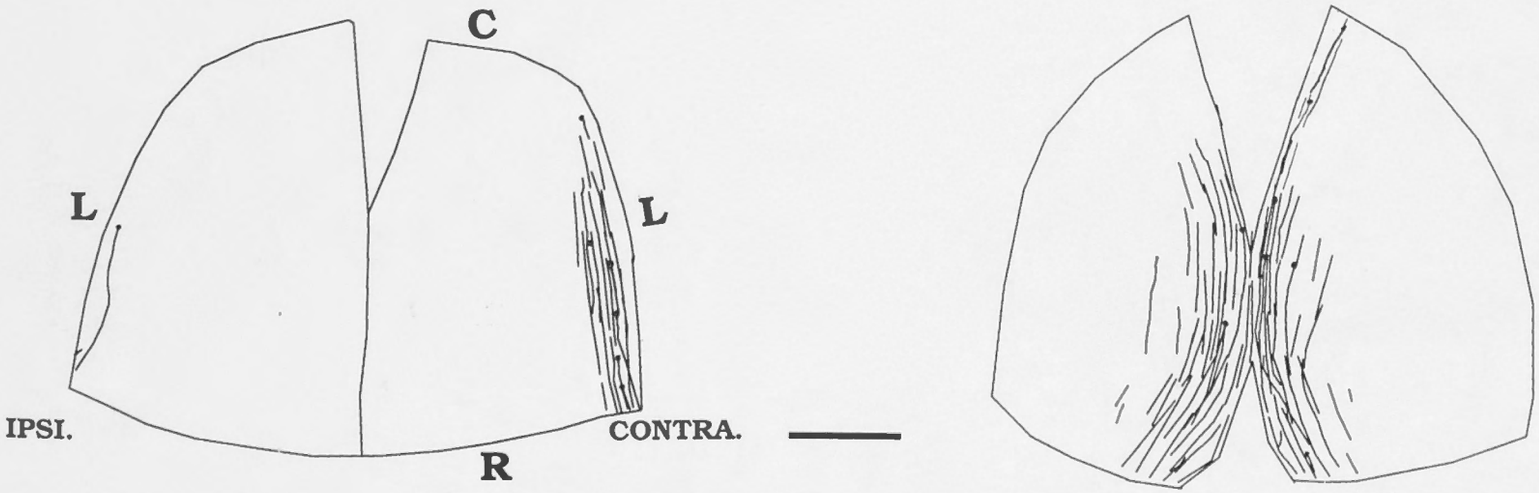


Figure 4.16 Fluorescent image of labelled axons in the contralateral SC at 28 days

Many labelled axons extend straight towards the caudal pole in a primarily rostrocaudal direction. Arrows indicate small side branches which are rarely seen. R: rostral; C: caudal. Bar: 100 μm.

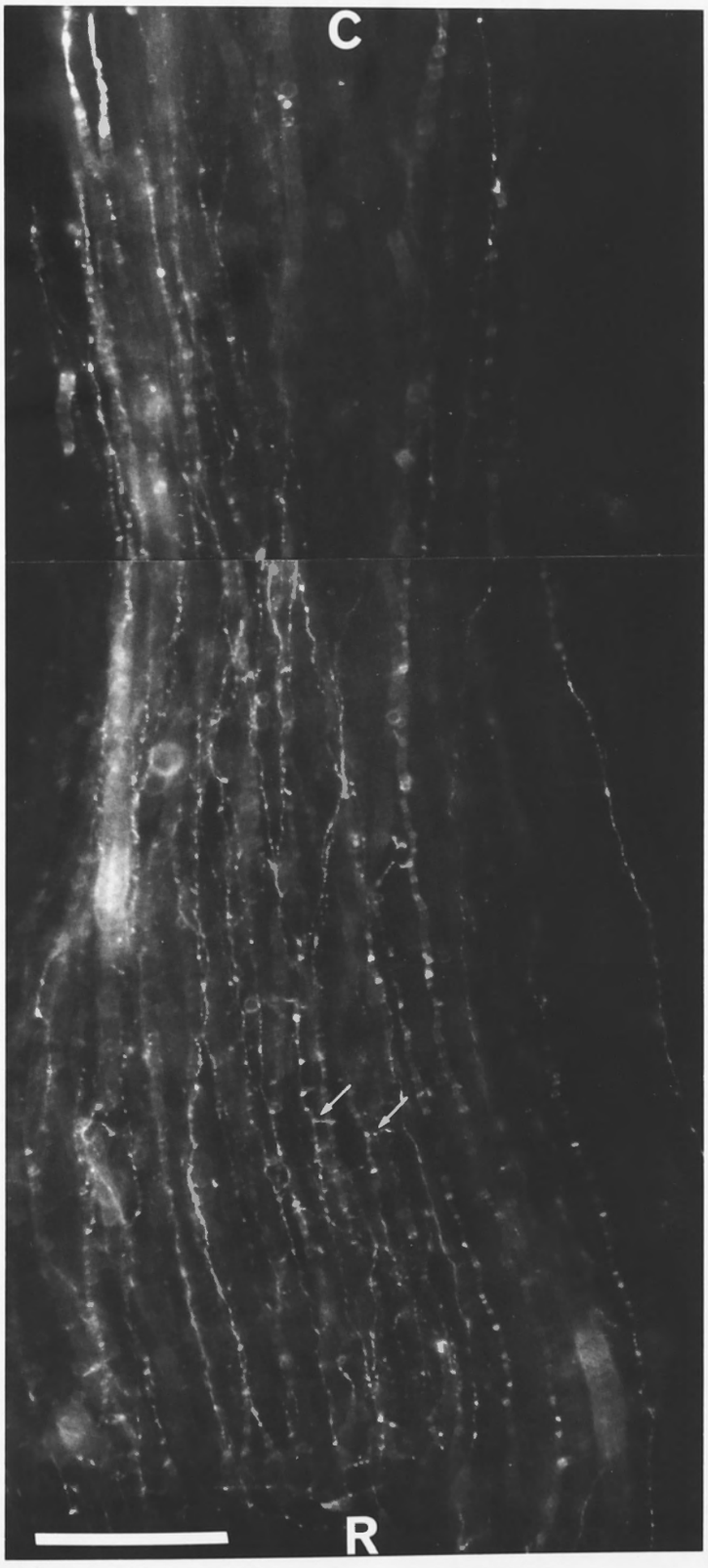
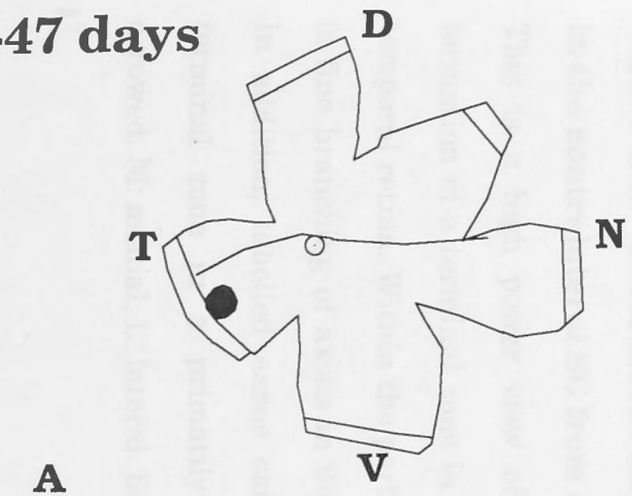
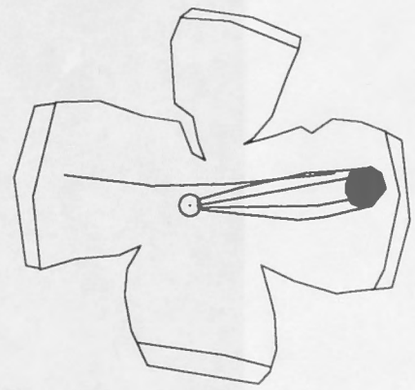

C
R

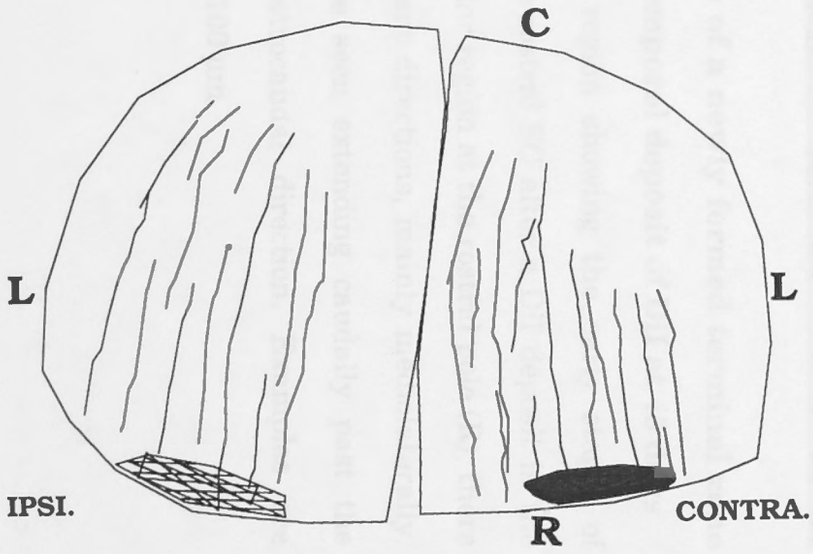
Figure 4.17 Camera lucida drawings of wholemounts of retina and the SC with temporal and nasal deposits at 41-47 days

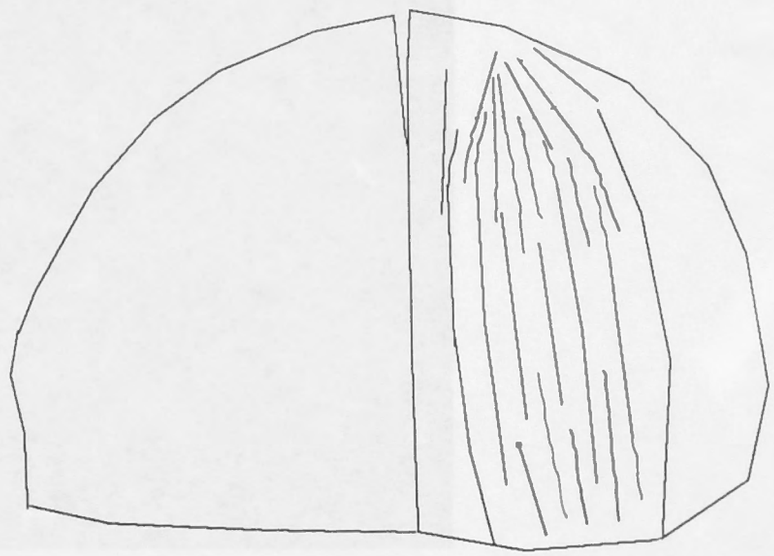
Conventions are the same as for figure 4.2 and 4.5. In addition, cross hatched area indicates the terminal zone in the ipsilateral SC. **(A):** A DiI deposit is positioned in the periphery of the temporal retina, slightly ventral. No axons can be followed from the DiI deposit site to the OD. Contralaterally, a terminal arborization appears at the rostral edge. It is elongated in the medial-lateral direction. Many axons can be followed past the terminal region. They extend caudally but stay approximately in the rostral two thirds of the SC. Growth cones are rarely seen. An approximate mirror image to that seen on the contralateral side is seen on the ipsilateral SC. A terminal zone in the form of a mediolaterally orientated band is at the rostral edge of the SC. Many axons are distributed caudally but confined to the rostral two thirds of the SC. Branching of axons outside the terminal zone and growth cones are rarely seen. **(B):** Labelled axons from nasal retina are distributed across the rostrocaudal extent of the SC. They turn towards each other and gather together at the far caudal pole but no terminal branching is present. Bars: 1 mm.

41-47 days









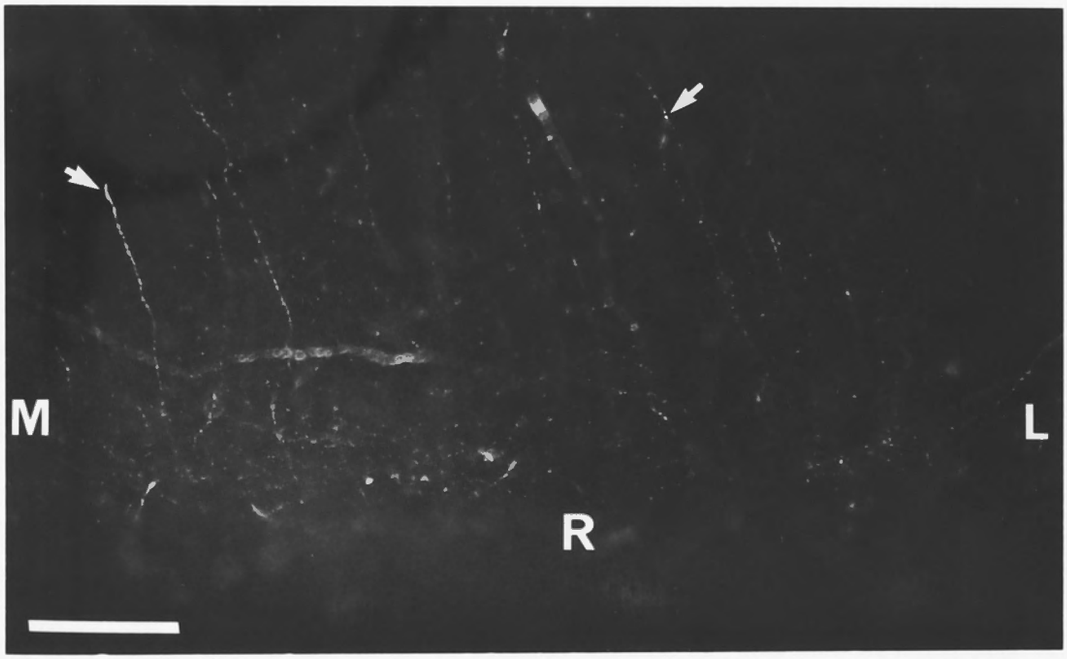


Figure 4.18 Fluorescent image of a newly formed terminal zone in the contralateral SC from a temporal deposit of DiI at 46 days

This is a high power view of a region showing the early stages of formation of a terminal zone in the rostral SC after a DiI deposit in the temporal retina. Within the particular region at the rostral pole (R), there is fine branching of axons in different directions, mainly mediolaterally. In addition, labelled axons can be seen extending caudally past the terminal zone in a primarily rostrocaudal direction. Examples are arrowed. M: medial; L: lateral. Bar: 100 μm.

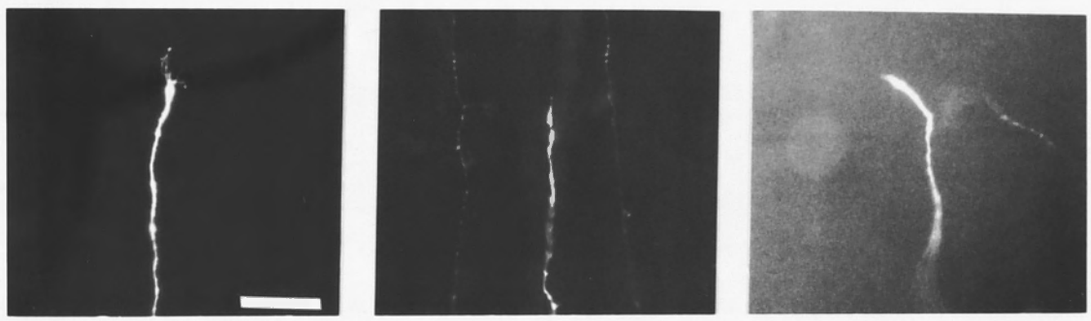


Figure 4.19 Fluorescent images of simple growth cones in the SC at 41-68 days

The morphology in this group of individual growth cones is defined as "simple". The growth cones are slender and rod-like. Filopodia are rarely seen. This type of growth cone is more common at this age than the complex growth cones seen earlier. Bar: 50 μm.

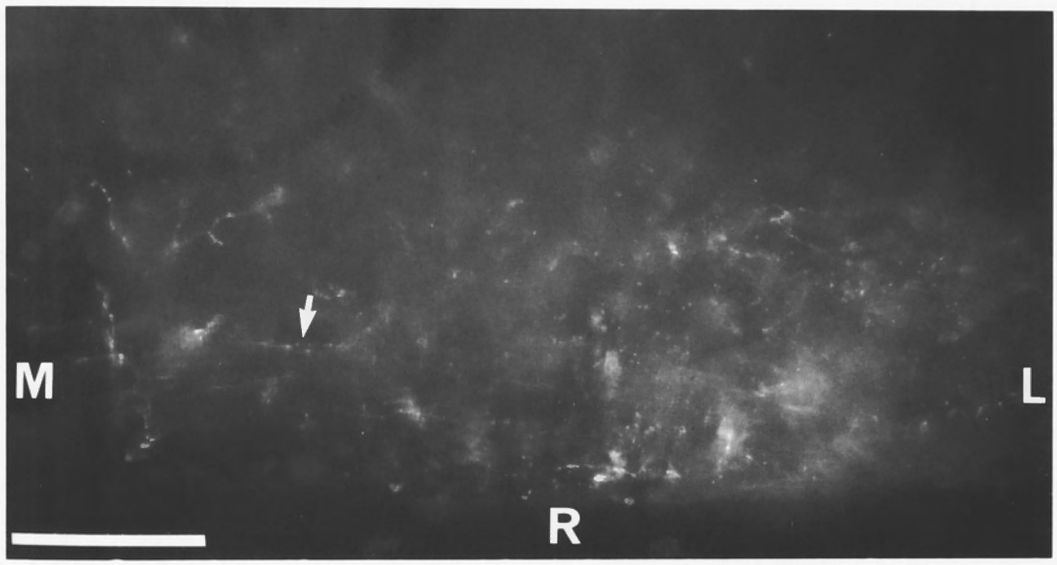


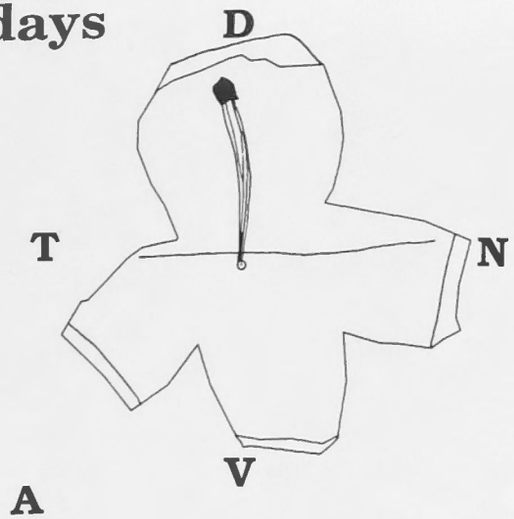
Figure 4.20 Fluorescent image of a terminal zone in the ipsilateral SC from a temporal deposit at 52 days

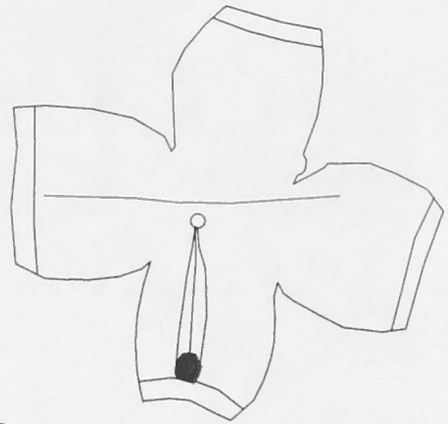
High power view of the terminal zone. Axon branching, which is less than that seen in the contralateral side, can be detected throughout this region, mainly in a mediolateral direction (arrow). Many of the fine branches are below the resolution of the microscope, resulting in a cloudy background appearance. R: rostral; M: medial; L: lateral. Bar: 100 μm.

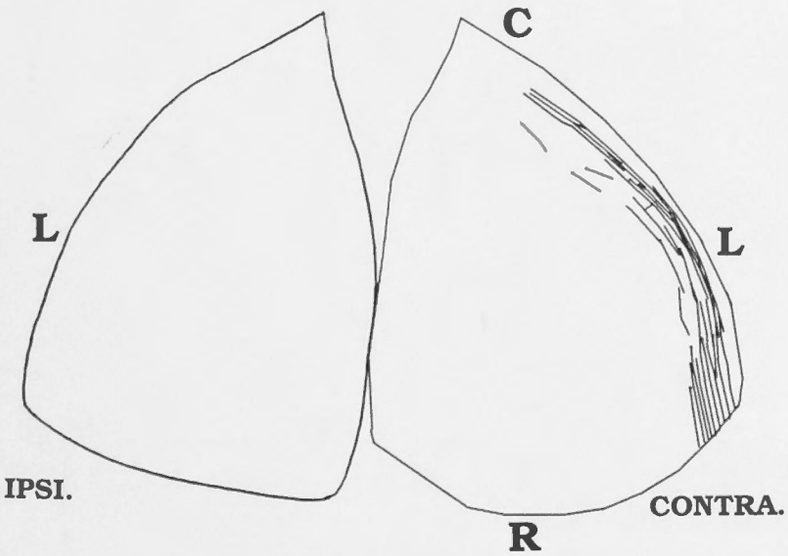
Figure 4.21 Camera lucida drawings of wholemounts of the retina and the SC with dorsal and ventral deposits of DiI at 41-47 days

Conventions are the same as for figure 4.5. **(A):** Many axons from the dorsal retina are seen along a narrow band in the lateral border of the contralateral SC, with more axons concentrated on the lateral edge. No terminal zone is seen. **(B):** Labelled axons, after a DiI deposit in the ventral retina, are distributed medially on both the contralateral and ipsilateral SC. Contralaterally, the axons extend rostrocaudally along the medial border and most of them are distributed on the medial edge. Growth cones and axon branching are uncommon. Fewer labelled axons form an approximate mirror image in the ipsilateral SC, where the axons are concentrated on the most medial border. A terminal zone is not present. Bars: 1 mm.

41-47 days







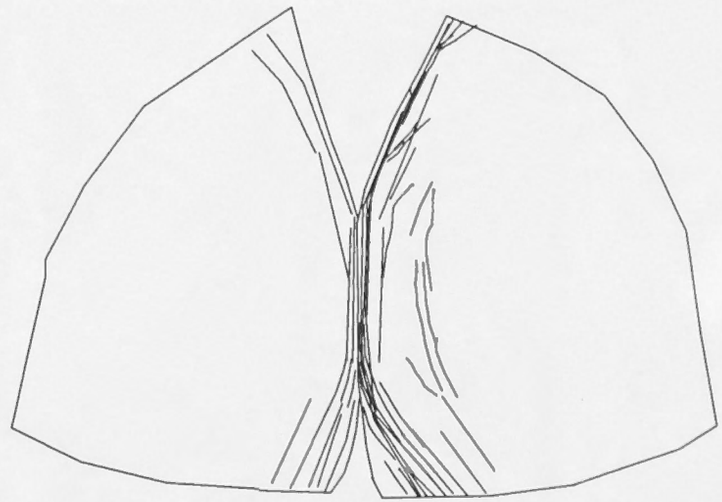


Figure 4.22 Fluorescent images of terminal zones after deposits in the temporal, nasal, dorsal and ventral retina at 52-55 days

(A): A terminal zone of temporal retinal ganglion cells in the contralateral SC is shown. Many fine beaded branches are distributed on a cloudy background. A denser region with more branching is seen in the centre of this region (arrow). The edge of the terminal zone is not sharp. **(B):** A terminal zone from axons in the nasal retina is shown. Fine, beaded branches are distributed throughout this particular region. **(C)** shows a terminal zone of axons from the dorsal retina. Many branches can be clearly seen around this particular zone. Axons passing through the terminal region extend in a primarily rostrocaudal direction along the lateral border of the SC. Photomicrograph **(D)** shows a terminal zone from the ventral retinal ganglion cells. Note that the terminal zones from the temporal and dorsal retina appear more mature, with more highly branched axons and intense fluorescent label. Bar: 100 μm.

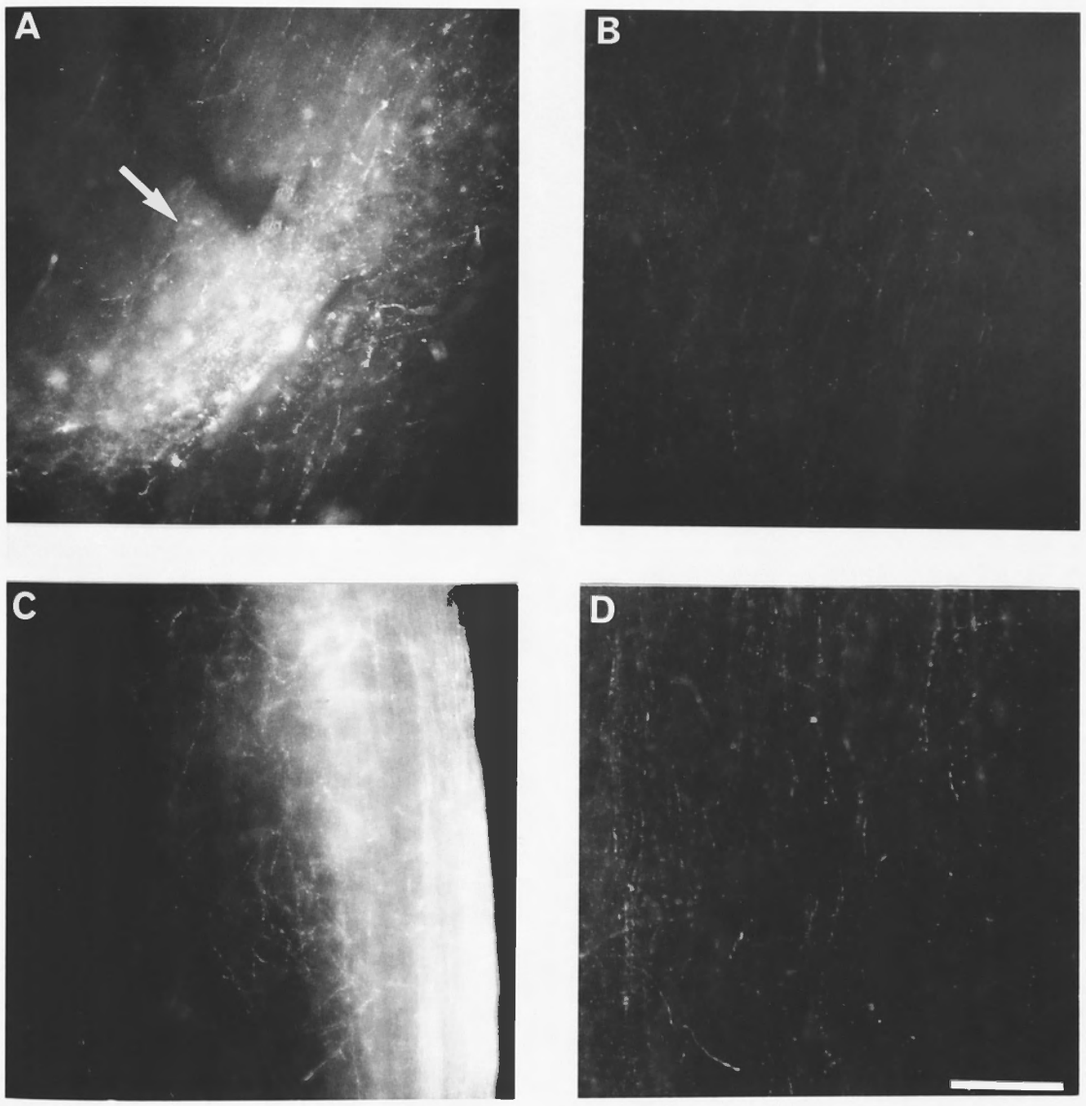
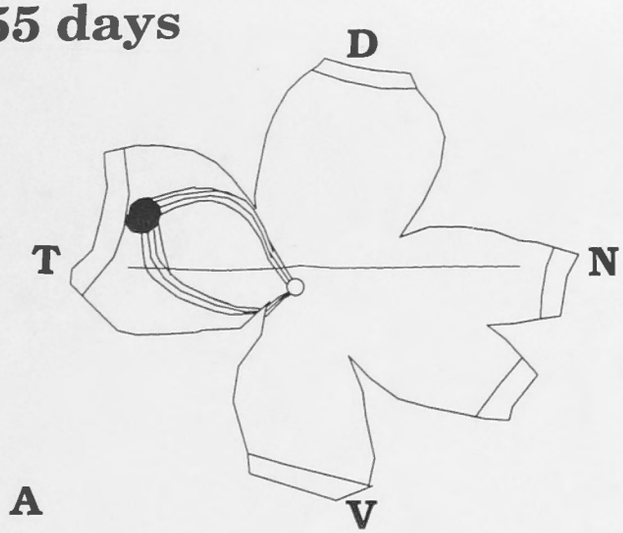

A
B
C
D

Figure 4.23 Camera lucida drawings of wholemounts of the retina and the SC with temporal and nasal DiI deposits at 52-55 days

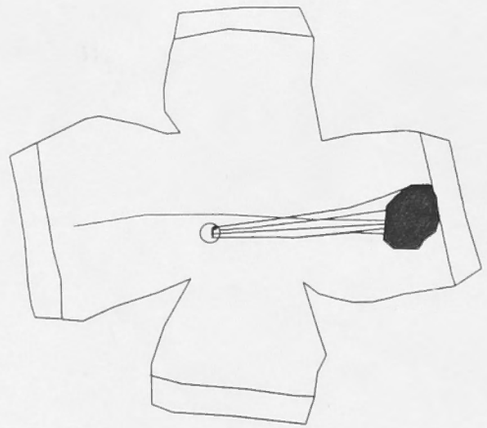
Conventions are the same as for figure 4.2 and 4.5 and 4.17. **(A):** A DiI deposit was placed in the periphery of temporal retina, slightly dorsal. Axons between the deposit site and the OD are labelled in two bundles. Consenquently, a terminal zone is positioned laterally in the rostral contralateral SC. Many labelled axons overshoot caudally pass the terminal zone. However, they are still confined to the rostrolateral region. A mirror image to the contralateral one is formed on the ipsilateral SC, in which a terminal zone is positioned in the rostral SC and many axons extend caudally pass the terminal zone, confined to the rostrolateral part of the SC. **(B):** A terminal zone formed by nasal axons is positioned caudally on the contralateral SC. Many axons coming from the rostral pole reach it, with a few axons passing further caudally to it. No labelling is detected ipsilaterally. Bars: 1 mm.

52-55 days

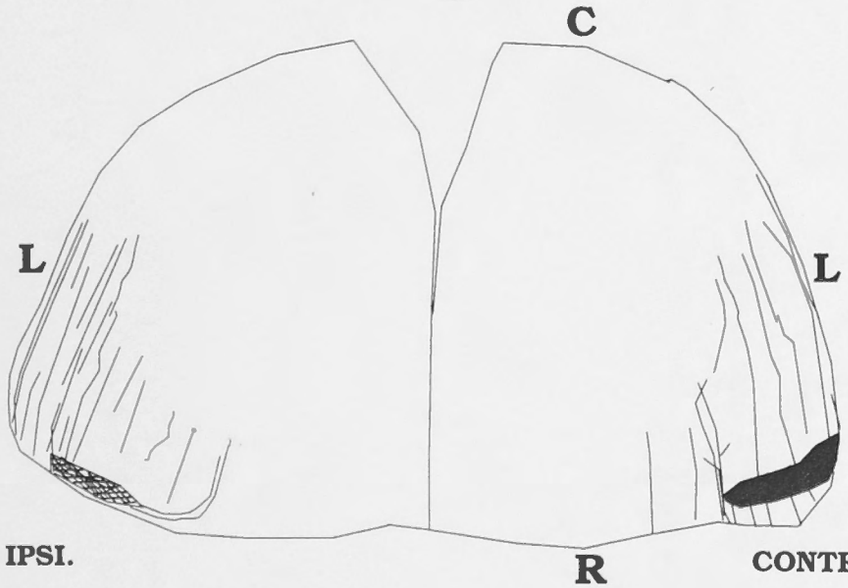
52-55 days



A



B



IPSI.

CONTRA.

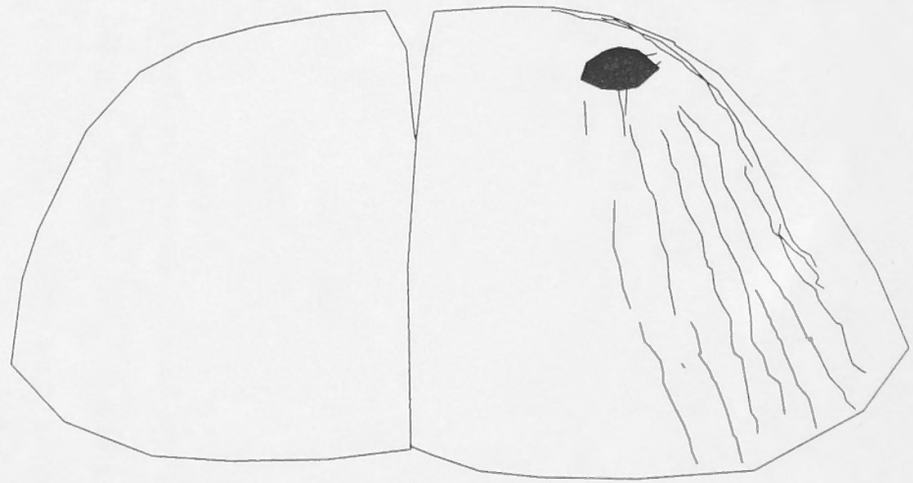
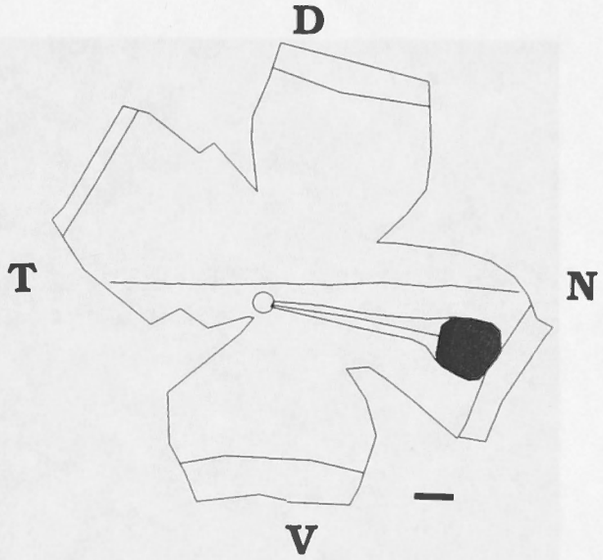
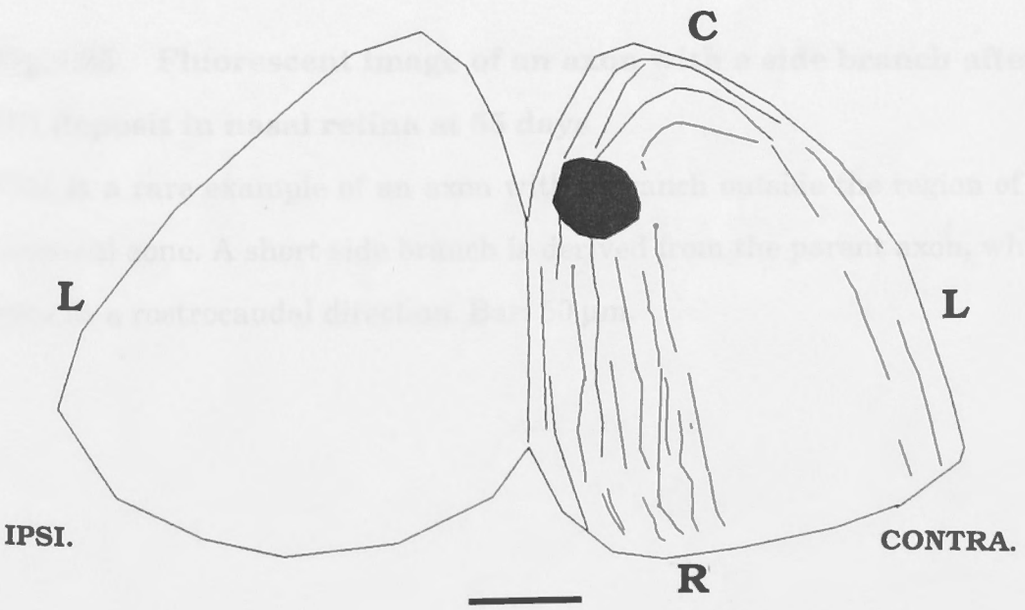


Figure 4.24 Camera lucida drawings of wholemounts of the retina and the SC with a deposit in the nasal retina at 55 days

Conventions are the same as for figure 4.2 and 4.5. DiI was placed in the nasal, slightly ventral retina. Consequently, the terminal zone is localized contralaterally in the mediocaudal SC. Labelled axons are distributed widely outside the terminal zone. They are rostrocaudally spread along the medial border of the SC. Some axons extend past the terminal zone caudally and at the caudal pole they turn around and extend towards the rostral pole along the lateral border of the SC. Bars: 1mm.

55 days





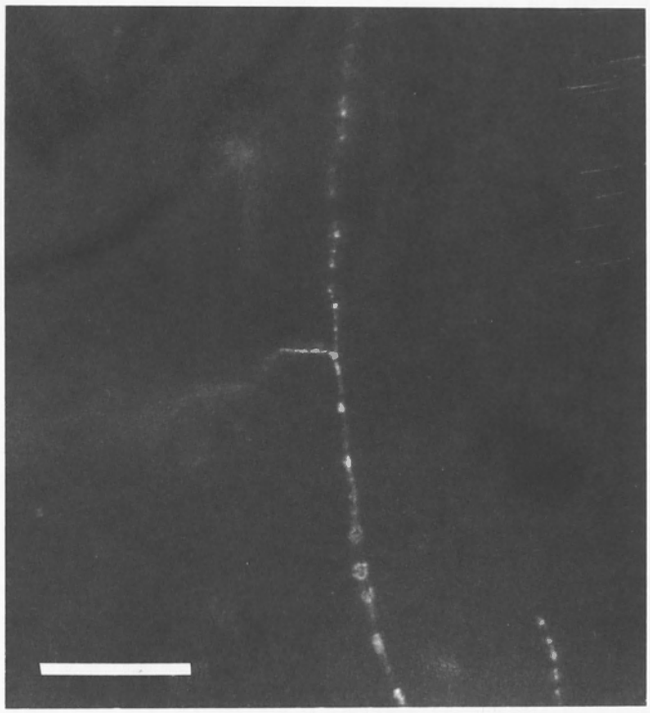


Fig.4.25 Fluorescent image of an axon with a side branch after a DiI deposit in nasal retina at 55 days

This is a rare example of an axon with a branch outside the region of its terminal zone. A short side branch is derived from the parent axon, which runs in a rostrocaudal direction. Bar: 50 μm.

Figure 4.26 Camera lucida drawings of wholemounts of the retina and the SC with dorsal and ventral DiI deposits at 52-55 days

(A): The terminal zone of the dorsal retinal ganglion cells is located in the lateral contralateral SC. Many axons approach it from the rostral pole and the axons overshoot caudally. They are confined to a band along the lateral SC with only one axon with a side branch seen outside the region. A very few axons are seen on the lateral edge of the ipsilateral SC. **(B):** The terminal zone of the ventral retinal ganglion cells is positioned medially in the contralateral SC. Axons are distributed outside the terminal region and extend caudally along a confined narrow area in the medial border of the SC, where there are more axons in the medial edge. In the ipsilateral SC, a few axons extend along the medial border but do not reach the far caudal pole. A terminal zone is absent. Bars: 1mm.

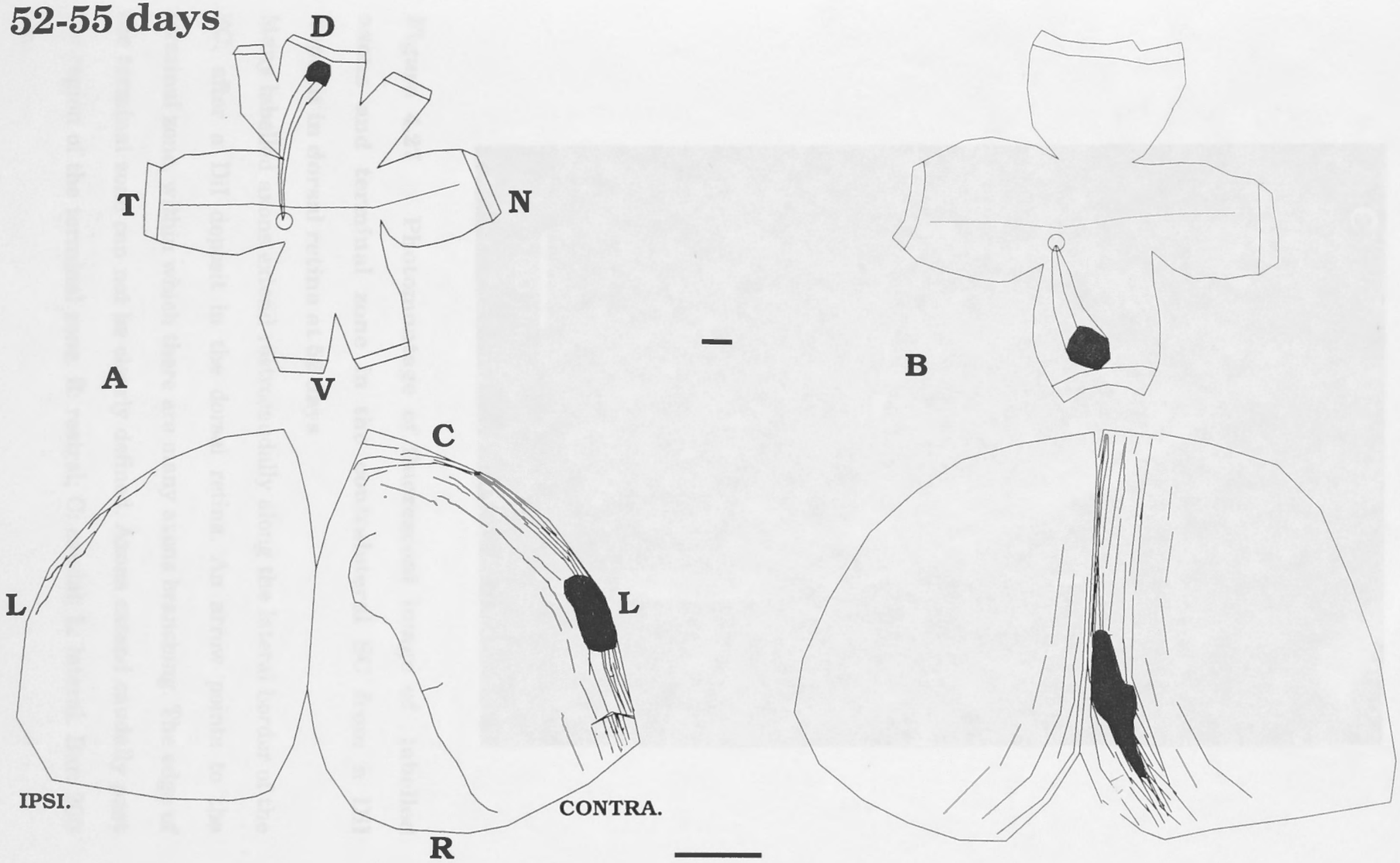

52-55 days
D
T
N
A
V
B
C
L
L
IPSI.
CONTRA.
R

Figure 4.27 Photomontage of fluorescent image of labelled axons and terminal zone in the contralateral SC from a DiI deposit in dorsal retina at 52 days

Many labelled axons extend rostrocaudally along the lateral border of the SC, after a DiI deposit in the dorsal retina. An arrow points to the terminal zone, within which there are many axons branching. The edge of the terminal zone can not be clearly defined. Axons extend caudally past the region of the terminal zone. R: rostral; C: caudal; L: lateral. Bar: 200 µm.

Figure 4.28 Camera lucida drawings of wholemounts of the retina and the SC with DiI deposits in the temporal and nasal retina at 61-68 days

Conventions are the same as for figure 4.2 and 4.5. **(A):** A clearly defined terminal zone from temporal ganglion cells is localized rostrally. A very few axons are distributed adjacent to this terminal region. **(B):** A terminal zone from nasal axons is present caudally. No axons are distributed outside the region. Bars: 1 mm.

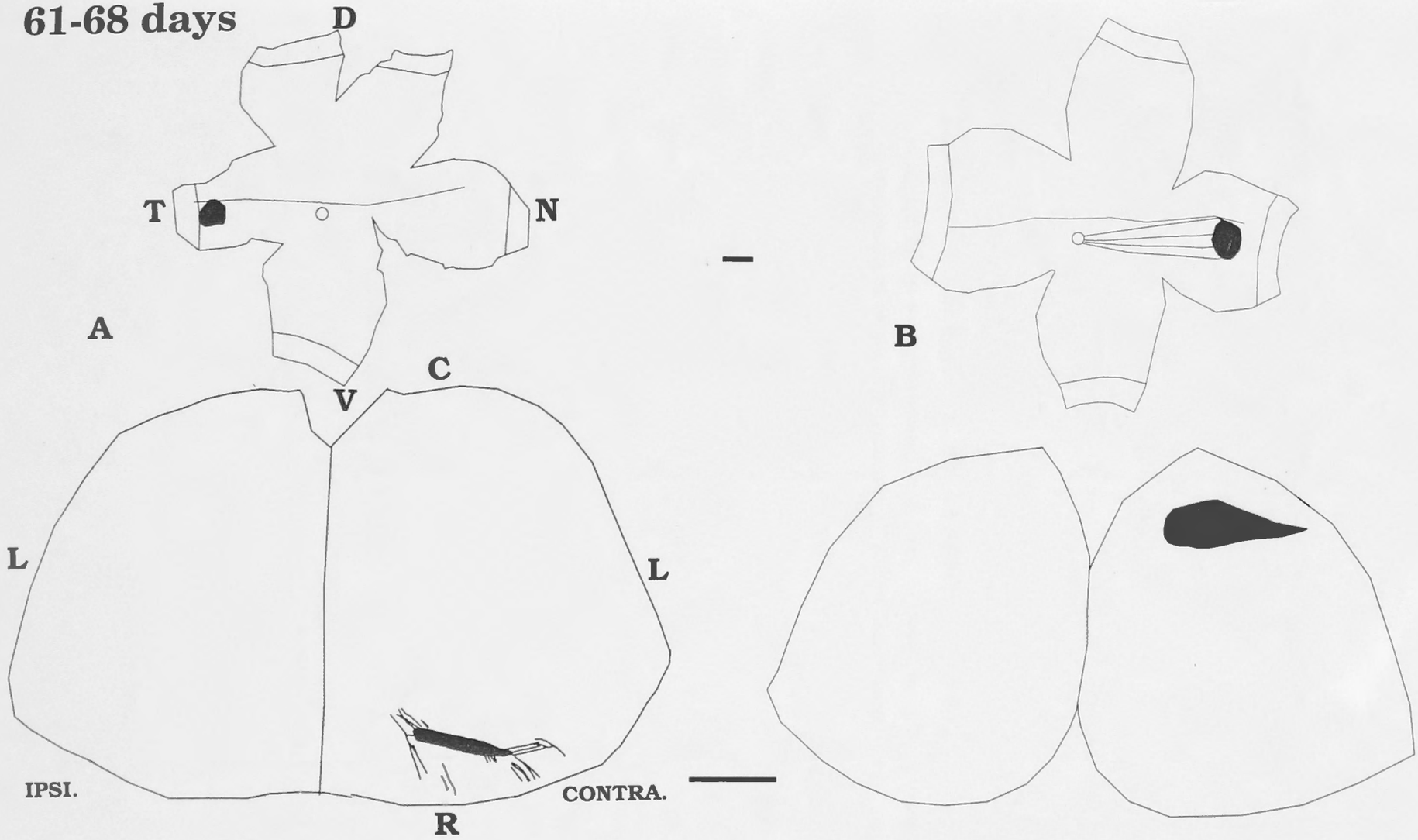

61-68 days
D
T
N
A
V
C
L
L
IPSI.
CONTRA.
R
B

Figure 4.29 Fluorescent images of terminal zones with DiI deposits in temporal, nasal and dorsal retina at 61-68 days

(A) and (B) shows terminal zones in the rostral and caudal SC after deposits in the temporal and nasal retina. The branching is extremely fine. Coarse branches are rarely detected. **(C)** shows a clearly defined terminal zone in the lateral SC after a deposit in dorsal retina. Here fine branching of axons can be seen over the region. A lower-power photomicrograph **(D)** shows the same terminal zone, which is focally positioned in the lateral SC. Widely distributed axons are absent. Bars: 100 μm.

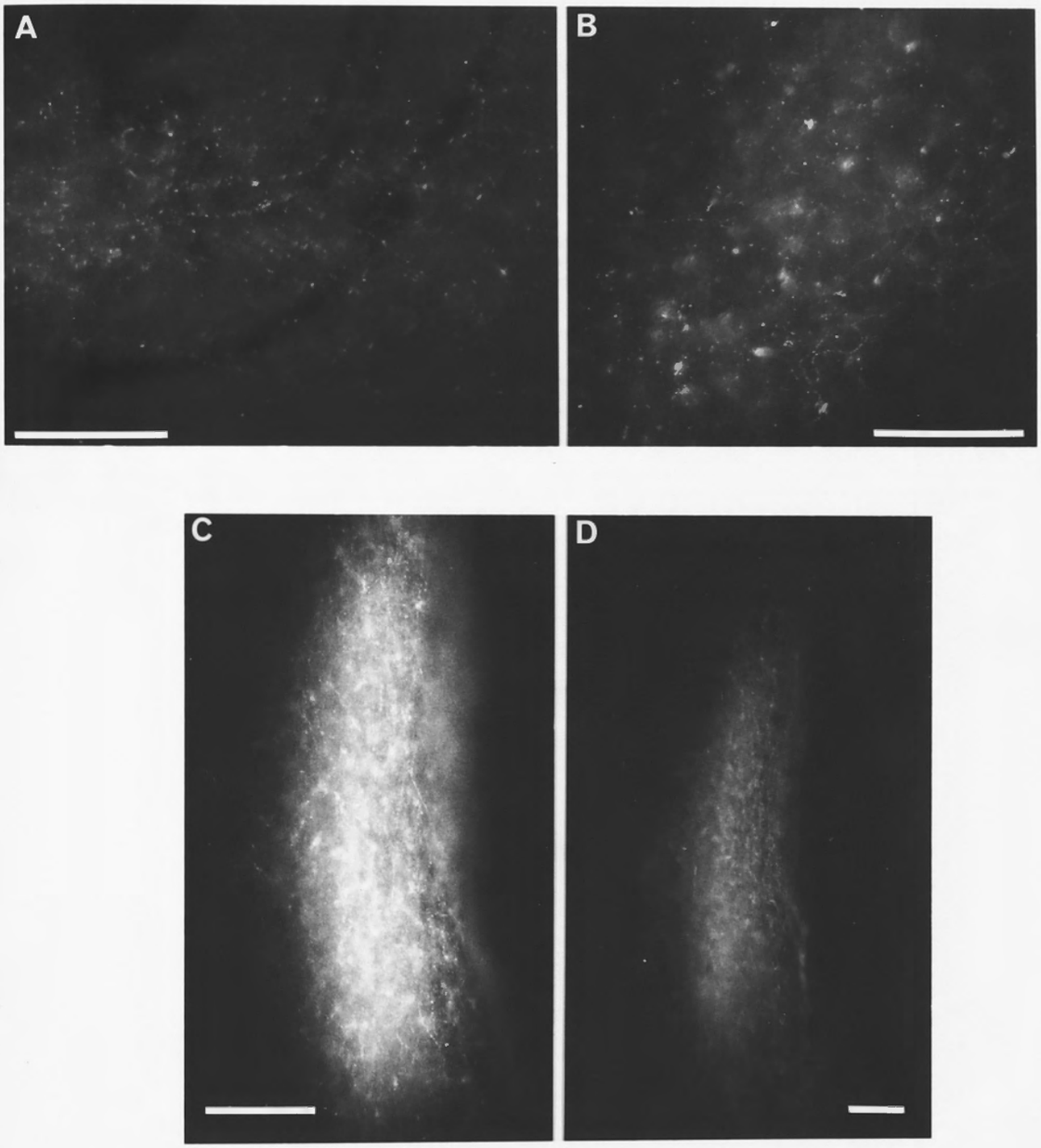

A
B
C
D

Figure 4.30 Camera lucida drawings of wholemounts of the retina and the SC with DiI deposits in the dorsal and ventral retina at 61-68 days

(A): A terminal zone from the dorsal retinal axons is localized laterally. A few axons can be followed from the rostral pole to the zone and a couple of axons can be seen caudally to it. **(B):** After a ventral deposit of DiI in the retina, a terminal zone is present medially. A few axons extend caudally past the terminal zone. One axon is seen more medially in the ipsilateral SC. Bars: 1 mm.

61-68 days

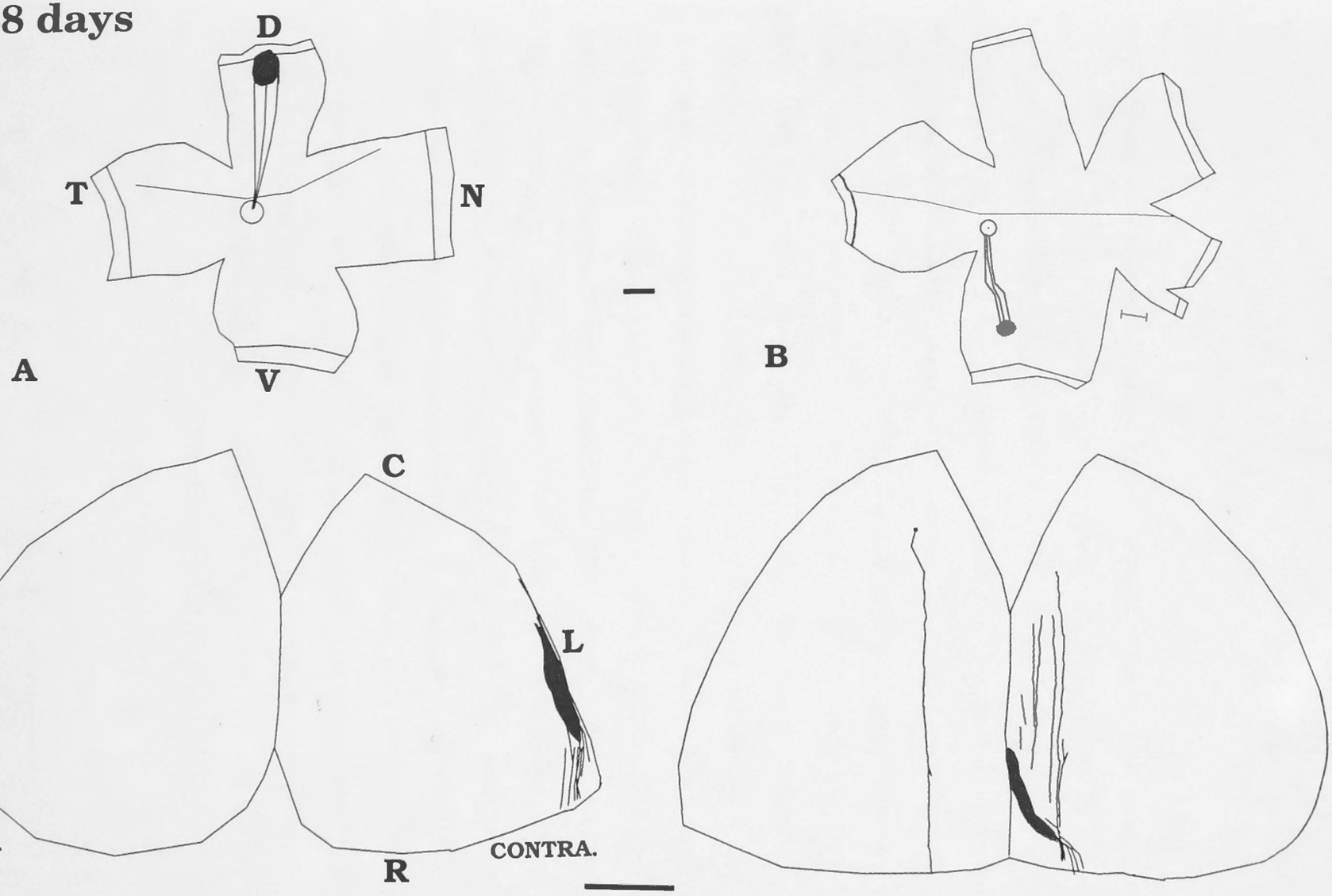


Figure 4.31 Coverage of retina by DiI deposits and coverage of the SC by labelled axons and by terminal zone

(A): Percentage of the SC covered by labelled axons. At no stage during development do axons from temporal, nasal, dorsal or ventral retina cover the whole of the SC. Axons from temporal retina are confined to more rostral SC, reaching a mean peak coverage of around 35% of the SC at the time when terminal zones begin to form from temporal retina, and falling thereafter. Axons from nasal retina reach a peak coverage of 65% at this time. Similarly, axons from ventral and dorsal retina are confined to lateral and medial SC respectively and reach a mean peak coverage of 20-34% of the SC at the time when axons from these retinal quadrants begin to form terminal zones. **(B): Percentage of the SC covered by terminal zones and absolute area of terminal zones.** Both the mean of percentage of the SC (dashed line) and the absolute size (solid line) covered by terminal zones from all retinal quadrants decreased with age. **(C): Percentage of the retina covered by DiI deposits**. The mean percentage coverage of the retina by the deposit of DiI was similar at all ages. **(D): Area of terminal zones from each retinal quadrant.** Size of terminal zones from different retinal quadrants decreased with age. T: temporal; N: nasal; D: dorsal; V: ventral.

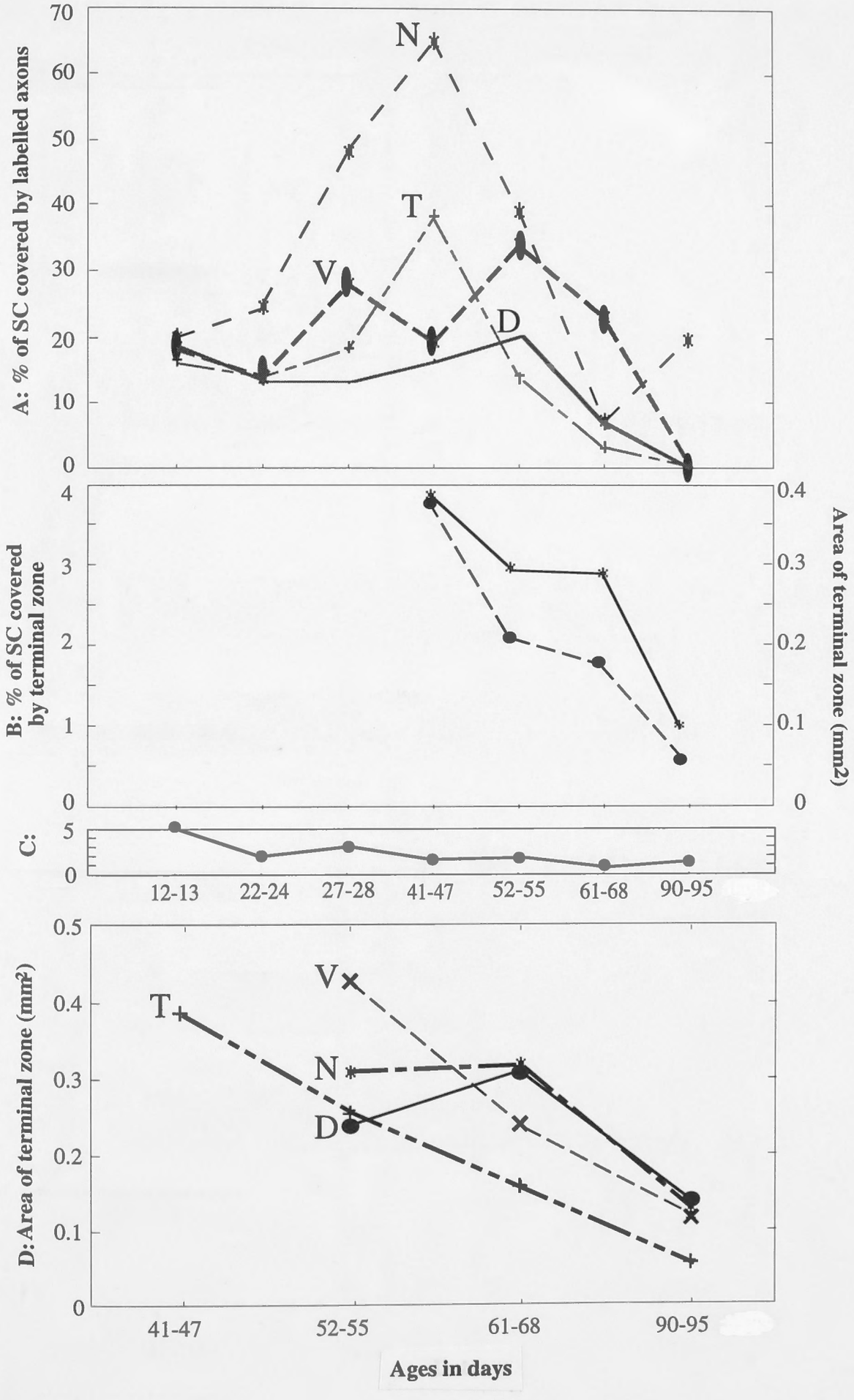
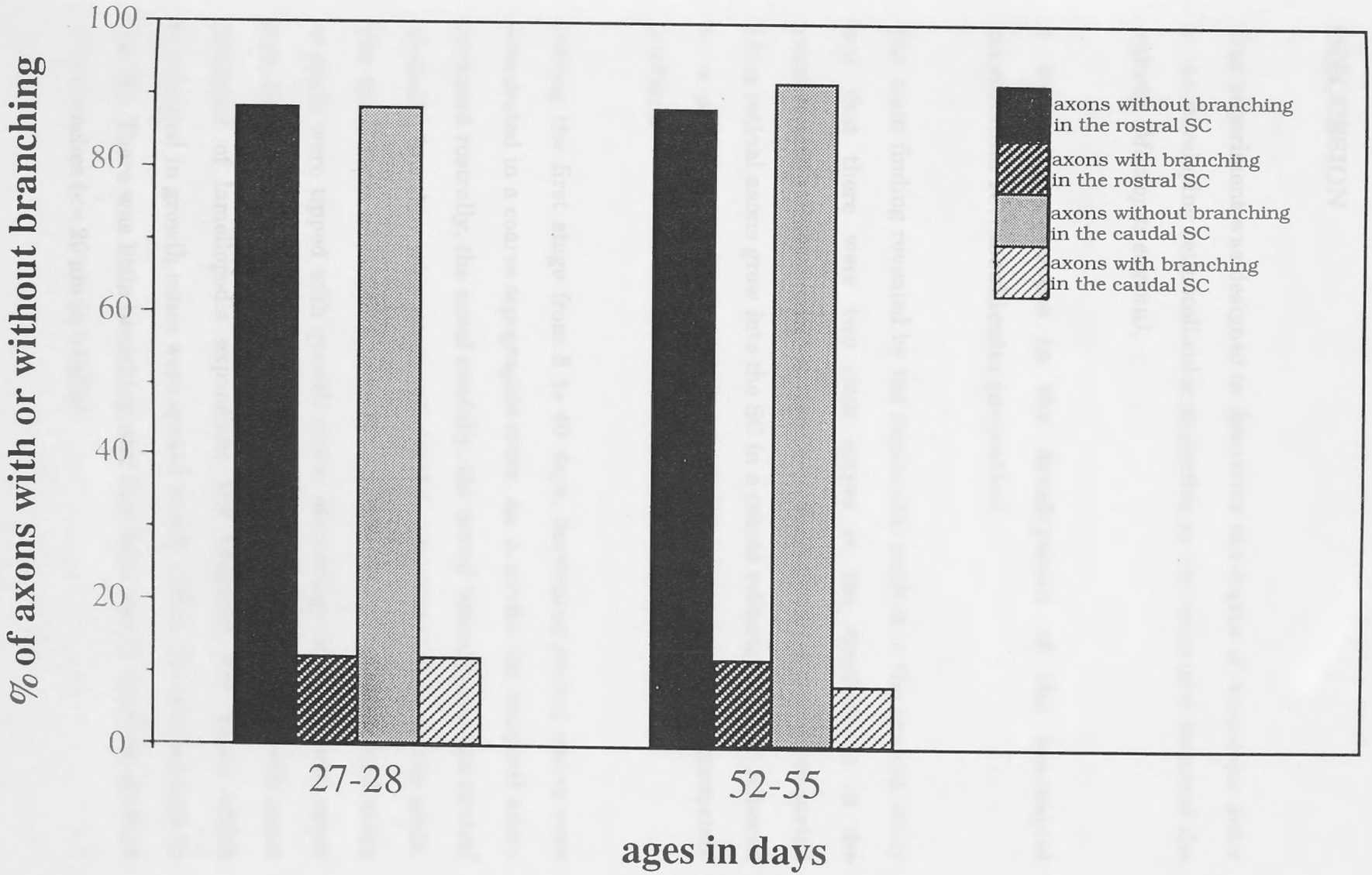

A: % of SC covered by labelled axons
70
60
50
40
30
20
10
0
N
T
V
D
B: % of SC covered by terminal zone
4
3
2
1
0
0.4
0.3
0.2
0.1
0
Area of terminal zone (mm2)
C:
5
0
12-13
22-24
27-28
41-47
52-55
61-68
90-95
D: Area of terminal zone (mm²)
0.5
0.4
0.3
0.2
0.1
0
V
T
N
D
41-47
52-55
61-68
90-95
Ages in days

Figure 4.32 Branching of nasal axons in the SC during development

Data is shown for early in development as axons grow into the SC (27-28 days) and around the time of formation of terminal zones (52-55 days). Nasal axons destined to form terminals in far caudal SC have few branches either rostrally in inappropriate territory or caudally in appropriate territory at either age.


% of axons with or without branching
100
80
60
40
20
0
27-28
52-55
ages in days
axons without branching in the rostral SC
axons with branching in the rostral SC
axons without branching in the caudal SC
axons with branching in the caudal SC

DISCUSSION

This experiment was designed to determine the degree of retinotopic order in the developing retinocollicular projection in the marsupial mammal the wallaby *(Macropus eugenii)*.

A two stage process in the development of the marsupial mammalian retinocollicular projection

The main finding revealed by the anatomical method in the present study was that there were two clear stages in the development of the contralateral retinocollicular projection in the wallaby: a protracted period when retinal axons grow into the SC in a coarse retinotopic order, followed by a period when terminals are formed in retinotopically appropriate positions.

During the first stage from 8 to 40 days, developing retinal axons were distributed in a coarse topographic order. As in adults, the temporal axons projected rostrally, the nasal caudally, the dorsal laterally and the ventral medially, but they were much more widely distributed than in the adult. The optic fibres advanced primarily in a rostrocaudal direction and many of them were tipped with growth cones. Morphology of the growth cones was defined as "complex" at the early stages, since those growth cones consisted of lamellopodia expansions and filopodia. The axons which terminated in growth cones were spread evenly within the labelled area in the SC. There was little branching and that seen was in the form of short side branches (<= 20 μm in length).

During the second stage from 41 to 95 days, retinocollicular projections underwent the formation of neuronal connections. Beginning at 41 days, retinal axons began to form their terminal zones in the correct topographic area. The arborization was first observed in the rostral SC, innervated by the axons from the temporal retinal ganglion cells. From this stage, the numbers of complex growth cones decreased and the lower numbers of growth cones seen were slender and rod-like. From 52 days, axons from all retinal quadrants formed their terminals in the topographically appropriate zones although the widely distributed labelled axons were still prominent. The terminals formed by the temporal and dorsal axons appeared more mature than those formed by the nasal and ventral axons, which correlates with the finding that axons from temporal and dorsal retina first reached the SC rostrolaterally (chapter 2 and 3). At 41-55 days when axons from all quadrants of retina began to form terminal zones, they reached a mean peak coverage of the SC and fell thereafter. From 61-68 days, clearly defined terminal zones appeared and there were much fewer labelled axons. From 90-95 days, discrete terminals were present.

Technical considerations

The fluorescent dye, DiI was used *in vivo* as an anterograde tracer in the present study. Honig and Hume ('86) developed a method to use DiI as a neuronal tracer and they first demonstrated that DiI can be used to label living neuronal cells. In living tissues (*in vivo*), DiI either diffuses laterally in the plane of the plasma membrane or due to membrane internalization, becomes incorporated in intracellular vesicles. Such vesicles can be transported by active axonal transport of the internalized membrane, which constitutes a very fast and effective staining over long distance (Godement et al., '87). Furthermore, by using flat-mounts of tissue (retina

and the SC) and computer aided neurotracing technique, it was possible to visualize long stretches of retinal ganglion cell axons labelled by DiI in their entirety and to easily reconstruct these single fibres over a large portion of their trajectory in the SC.

In this experiment, DiI was directly applied to the retina, to demonstrate the exact identity of retinal axons with regard to the ganglion cells of origin. A solid material, gelfoam impregnated with DiI, was inserted into retina, since it was easier to produce a focalized deposit than by an injection of DiI in solution. There was no evidence that DiI from labelled axons spread to other axons. In addition, after carefully scanning the retina in all cases, ectopic labelling sites in the retina were not detected. Thus, the findings are regarded to be based on directly labelled axons arising from a focal, well-defined retinal placement of DiI. Absence of direct, axonal transfer was also supported by the results in the next chapter, in which labelled axons were found to be positioned retinotopically as groups in the optic nerve. In contrast, axons labelled after a focal DiI deposit in the periphery of the retina *in vitro* which could be followed in a discrete bundle in the retina resulted in labelled axons spread over the whole nerve near the optic nerve head and no appearance of retinotopic order in the SC (unpublished data). Labelled axons *in vivo* could be traced along their intraretinal course from the deposit site to the optic disc. No labelled axons were traced retrogradely to parent ganglion cells scattered widely over retina outside the deposit site, which would indicate axonal transfer followed by retrograde transport to parent ganglion cells.

Mostly, the DiI deposits were made into the peripheral retina of each quadrant, in order to avoid the possibility of labelling axons passing through the deposit site from more peripheral retina. However, the results

in the rat showed that there is a possibility that axons from central retina probably reach the SC before those from peripheral retina and they may differ from peripheral axons in their targeting behaviour (Morest, 1970). As well, axons arising from ganglion cells at the peripheral margin of the retina in the rat had a greater tendency to mistarget along the rostral-caudal SC axis than those from more central regions (Yhip and Kirby, 1990). Thus, a few deposits were made centrally into the retina in the present study, in order to compare with the results from the peripheral dye deposits. In these cases, retrograde labelling of axons that passed through the deposit site and arose from retinal ganglion cells peripheral to the deposits was not observed. The reason for this is not known. This was observed in only a few cases after central deposits in rat (Simon and O'Leary, '92a). General targeting behaviour of axons arising from more central located retinal ganglion cells was found to be the same as that of axons arising from peripheral retina.

Comparison with previous work in the wallaby

Results obtained from anterograde labelling of HRP in the developing wallaby (Marotte,'90) complement the present finding. Discrete retinal lesions were made and the remaining retinal projections to the SC were traced. The time when the filling defect in the contralateral SC was first detected at 43 days is correlated with the time when the terminal arborizations were first formed in the similarly retinotopically appropriate area at 41-47 days in the present study. This suggests that a point to point retinocollicular connection begins to be formed around this time. Marotte's study ('90) also showed discrete terminal zones being present retinotopically by 90-95 days. The retinal projection to the contralateral SC became mature by 93 days, since the borders of defects in the SC after a lesion of retina were sharp and terminal label was completely absent

within the defect. A more recent study (Marotte,'93), using HRP as a retrograde tracer placed in the caudal pole of the SC also correlates well with results observed in the present experiment. There was a patch of appropriately positioned retinal ganglion cells with inappropriately positioned cells between this dense patch in the nasal peripheral retina and the optic disc and also dorsal and ventral to it from early times (22 days). These errors reached a peak at 45 days of age, decreased to low level by 63 days with a high proportion of labelled cells within the patch and by 92 days were absent. Correspondingly, in the present study, at 41-47 days when terminal zones began to form in the retinotopically correct position, the distribution of labelled axons from the temporal and nasal retina reached a peak in the SC, followed by the disappearance at 61 days, of many of the initially more widely distributed axons and the presence of defined terminal zones. By 92 days, both studies suggested that retinotopy was judged to be precise since either a discrete patch of heavily labelled cells in nasal retina or a discrete terminal zone in the SC was present. Marotte's ('93) result that the inappropriately projecting ganglion cells do not originate from the entire retina but from regions adjacent to the appropriate region such as dorsal and ventral, also corresponded to the observations in the present anterograde tracing study. When DiI was deposited along the horizon of the eye, depending on whether it was slightly dorsal or ventral, the labelled axons reached their targets in the rostral or caudal SC along the lateral or medial border. The findings suggest that the retinal ganglion cells along the temporal-nasal axis might only have a loosely defined pathway in the SC and can overlap with the pathways taken by adjacent ganglion cells coming from the dorsal and ventral retina, to approach the appropriate target during early development. Also the finding that inappropriately positioned cells outside the patch of appropriately positioned cells in nasal retina only extended in

a nasotemporal direction as far as the vicinity of the optic disc fits with the present finding that temporal axons did not extend to the far caudal SC at any stage. Further, it was also shown in Marotte's study ('93), that during development the number of inappropriately projecting cells in the temporonasal dimension was larger than that in the dorsoventral dimension in retina. This finding correlates with the finding in the present study, that coverage of labelled axons in the SC from dorsal and ventral retina was more defined than that formed from temporal and nasal retina.

In addition, more evidence confirming the two stages in the formation of the marsupial mammalian retinocollicular projection has come from a concomitant electrophysiological experiment (Freeman, '92; Mark et al., '93b). A response to stimulation of the optic nerve head was first recorded and a negative potential on the surface reversed to positive deeper at the rostral pole of the contralateral SC at 42 to 45 days. The onset of synaptic activity corresponds with the first appearance of terminal arborizations of temporal retinal axons in their appropriate retinotopic position. Prior to this when axons were distributed coarsely, no electrical activity could be recorded. By 55 days the area of the SC from which an evoked potential could be recorded had increased and by 87 days the reversing evoked potentials with some inflexions characteristic of the mature form could be recorded from the entire surface of the SC, which coincides with the time when discrete terminal zones start to appear. Therefore, innervation of the contralateral SC by the optic ganglion cells was found to be composed of two separate processes: firstly, a massive ingrowth of fibres which were disposed in a rough retinotopic order without evidence of synapse formation or nerve conduction; and secondly specific synaptic connections with the emergence of functional transmission.

Projection to the ipsilateral SC

Topographic order in the retinal projection to the ipsilateral SC during development was also demonstrated in the present study. At 12 days, the retinal projections to the ipsilateral SC were observed only after labelling ganglion cells in the dorsal and temporal retina. Labelled axons, which were extremely few in number, were followed laterally and rostrally in the ipsilateral SC, obeying the same retinotopic order as in the contralateral SC. Indeed, at all stages from 12 to 55 days, labelled axons seen in the ipsilateral SC were found to obey the same topographic plan as seen on the opposite side. After temporal deposits of DiI from 22 days and ventral deposits thereafter, the ipsilateral projection to the SC was more or less a mirror image of the contralateral one about the medio-lateral axis, in more rostral and medial SC, respectively. In contrast, DiI deposits in the nasal and dorsal retina over this time labelled either no or very few axons, but when present they were in topographically appropriate regions.

The results from retrograde labelling of axons from the SC at around 12 days (chapter 3) showed that cells arose not only from the dorsal and temporal region of the retina as found in the present study, but also from the other retinal regions. However, cells were extremely sparse and often in more central retinal regions while deposits of DiI were peripheral and so could easily have missed labelling the sparsely distributed cells in nasal and ventral retina. The results from the present study show that the major ipsilateral projection comes from the temporal and ventral retina from relatively early in development. This fits well with the findings reported in chapter 3 where ganglion cells supplying the retinal projection were found to be concentrated in the peripheral temporoventral retina with only a few cells scattered throughout the rest of the retina from 16 days. In this study

(chapter 3) no cells were found outside the temporoventral crescent after 41-46 days, while in one case in the present study a very few axons were labelled after a dorsal deposit after this time, suggesting that the latter method may be slightly more sensitive in detecting sparse projections. In previous experiments on the wallaby, using small retinal lesions and tracing the remaining retinal projection with HRP, the retinotopy of the mature projection to the ipsilateral SC was not able to be determined due to its sparse nature (Flett et al., '88). Early in development at 43-48 days when filling defects after retinal lesions could first be detected, only temporal and temporoventral lesions produced filling defect ipsilaterally (Marotte, '90). This is similar to the present results.

From 41-55 days, even though the retinal projection was extensive (chapter 2), the only terminal zones observed were formed by temporal axons in the rostral SC. However, an electrical response to stimulation of the optic nerve head could not be recorded ipsilaterally in the rostral SC at the same time (Freeman, '92). In fact, no functional connections ipsilaterally could be detected during development (Freeman, '92) or in the adult (Mark et al., '93a). From 61 days, the ipsilateral projection was hardly detected in the present study although there is an ipsilateral projection revealed by HRP labelling from the eye in the mature projection (Flett et al, '88). This could be explained by the fact that ganglion cells projecting to the ipsilateral SC at this age may be very sparse or may be concentrated in a small region, or both. A small deposit of DiI in the periphery of the temporoventral crescent of retinas may be inadequate to label ganglion cells projecting ipsilaterally. The anatomical finding, combined with the electrophysiological evidence of lack of functional connections suggests that the ipsilateral retinocollicular projection may be vestigial in the wallaby.

Formation of terminal zones

Ganglion cells in the periphery of temporal or nasal retina terminate in the rostral or caudal pole of the SC, at varying medial-lateral positions depending on the location of the retinal cells along the dorsal-ventral axis of the retina. Correspondingly, ganglion cells in the periphery of dorsal or

ventral retina terminate in the lateral or medial border of the SC, at varying rostral-caudal positions depending on the location of the retinal cells along the temporal-nasal axis of the retina. There were a number of possible ways in which the formation of discrete terminal zones may have occurred in the wallaby. First, as in rat, hamster and mouse (Schneider et al., '85; Sachs et al., '86; Jhaveri et al., '91; Bhide and Frost, '91; Simon and O'Leary, '92a), the terminal arborization could have been elaborated from one of the side branches along the parent axon which passed further caudally, followed by the subsequent loss of the axon distal to the terminal zone. This is considered possible in the wallaby as there were about 10% of axons with side branches early in development. Also considered likely is that axons passing through their area of termination with no side branches (the majority) could be induced to elaborate a terminal by a signal from the SC (see below), followed by loss of the more distal axon. Axons could also aborize at the tip to form terminals as they reached the topographically correct place, with other axons extending further caudally. Those axons forming terminal zones in this way may extend more slowly in the SC, or may be directed by a specific message from parent cells in retina or from collicular cells to remain in their correct targeted area prior to forming a terminal zone, rather than extending further caudally (see below). Alternatively, the axons may be late arriving axons that have just reached their target, since retinal ganglion cells are still likely to be extending axons into primary visual centres around the times of terminal connection (Spira and Marotte, '89). A pattern of forming terminal zones was reported in rat (Simon and O'Leary, '92a,b), in which a proportion of the axons labelled from each retinal region reached their region of termination by changing their trajectory along the rostral-caudal or medial-lateral axes of the SC. However, no evidence found in the wallaby supported this pattern of terminal formation. Lastly, removal of widely distributed axons could

also be achieved by cell death. Retinal ganglion cell death was found in wallaby to begin around 30 days and extend beyond the period of axon loss (Spira and Marotte, '89).

The possible mechanisms controlling the formation of retinotopic projections to the SC in the wallaby

Understanding of the mechanisms controlling the formation of spatially ordered connections between retinal ganglion cells and the colliculus is one of the major goals in the present study. From the work over the past several decades, mechanisms that may contribute to the retinotopic connections in the SC (the homologue of the optic tectum in non-mammalian vertebrates) can be classified into the several models. These include specific interactions between optic axons and tectal cells (Sperry,'44, '63; Bonhoeffer and Gierer, '84; Gierer, '87), pre-ordering of axons in the visual pathway such as optic nerve, direct or indirect axon-axon communication in the target area (Horder and Martin, '78; Willshaw and von der Marlsberg, '79; Bodick and Levinthal, '80) as well as functional tests of correct neighbourhood relations by impulse activity (Willshaw and von der Marlsberg, '76; Constantine-Paton and Law, '78; Whitelaw and Cowan, '81; Meyer, '83; Schmidt and Eisele, '85; Fraser and Perkel, '89; Schmidt and Tieman, '89; Schmidt, '90; Constantine-Paton et al., '90). These mechanisms are not mutually exclusive and no single mechanism can account for all facts in map formation.

Pre-ordering of axons in primary visual pathway. In the present study on the wallaby, one possible hypothesis to explain at least in part the initial development of coarse retinotopic order in the first stage could be a preordering model, in which the neighbourhood relationship of the retinal

ganglion cells is maintained in the optic pathways and into the SC. Ganglion cell axons from different retinal region may be guided towards their targets along relatively exclusive pathways by the conformation of the underlying tissue planes that they encounter. This possibility is supported by the findings in the next chapter: As labelled axons from each region of retina exited the eye to enter the optic nerve, they were still grouped together and retinotopically organized. The retinal axons may then retain their neighbourly relations throughout the rest of the visual passage and be introduced to the matched parts of their target, the SC. This idea is consistent with the finding that separation of dorsal and ventral axons was found in the optic tract as they approached the SC and the dorsal and ventral axons entered appropriately the lateral and medial SC respectively. However, the preference for temporal axons to occupy the rostral SC can not easily be accounted for in this model. Nasal and temporal axons both entered the SC across the width of the tract. This is considered futher in the next section.

Neuronal specificity. The results in the present study also strongly suggest that a topographically correct projection for retinocollicular axons in the wallaby is achieved by interactions between spatial markers carried by invading retinal axons and spatial markers in the target tissue, the SC. Theoretically, the development of this retinocollicular projection can be explained by the chemoaffinity hypothesis (Sperry,'44, '63), which was originally formulated in relation to the connection between the eye and the brain in the amphibian visual system and was based on the concept of neuronal specificity. This specificity was postulated to be essentially cytochemical in nature and to underlay the selectivity with which the ganglion cells would form synaptic connections in the tectum. The chemospecificity was consequently advanced in the form of a gradient

theory that postulates the existence of graded spatial markers on retinal axons and tectal cells (Sperry, '63; Fraser, '80; Fraser and Hunt, '80; Bonhoeffer and Geirer, '84; Gierer, '87; Walter et al., '90; Baier and Bonhoeffer, '92). Directional cues may also guide retinal axons by graded distribution of markers along the pathway from optic nerve to the SC (Sperry, '63).

The establishment of the coarse topographic map of retinal ganglion cell axons in the SC at the first developmental stage, in which optic axons growing out from the temporal retina remained in the rostral SC and axons from the nasal retina grew caudally, are likely to depend on such markers. This idea is supported by evidence of recognition of tectal cell membranes by retinal axons and the graded distribution of a molecule with position-encoding properties in tectal tissue from *in vitro* studies on chick (Walter et al., '87 a,b, '90), fish (Vielmetter and Stuermer, '89), frogs (Johnston and Gooday, '91), mouse (Godement and Bonhoeffer, '89) and rat (Simon and O'Leary, '92c). A preference for temporal axons to grow on rostral tectal membranes has been shown in these species. The behaviour of the temporal axons is postulated to be caused by a 33 kd repulsive guiding molecule (RGM) present in greater amounts on caudal than on rostral tectum (Stahl et al., '90). Other studies on chicken tectum (Trisler and Collins, '87; Trisler, '90) also indicated that there is a graded distribution of cell surface-associated proteins called TOP_{DV}, a 47 kd molecule and TOP_{AP}, a 40 kd molecule, with complementary gradients in the retina. How these molecules actually serve the roles suggested by their distribution remains to be tested, although TOP_{DV} within the retina was found to have an effect on growth cone persistence and synaptogenesis (Trisler et al., '86; Trisler, '90).

The delay between the arrival of optic axon in the SC and the initiation of the formation of terminals in the SC in the second stage strongly suggests that more specific spatial markers appear in the SC to give a precise and specific signal for formation of terminal arborizations in retinotopically correct regions. One way in which this could happen is that optic axons of the retinal ganglion cells which reach the SC in a coarse retinotopic order, carry with them the "specificity" characteristics of the parent cells. The retinal axons would recognize the information, in the form of molecules on the cell surface, given by their appropriate opposite number amongst the collicular cells. Thus, retinal axons could selectively and exclusively connect with the cells and form synapses. Axons that obtained no message from their target cells would disappear.

The appearance of the specific guidance molecules presumed to be derived from the target field at the time of terminal formation, could explain the morphological changes of growth cones at the second stage of development of the retinocollicular projection of the wallaby. During early development, axons tipped with growth cones were seen at all levels of the region where labelled axons were distributed. They had more complex shape than the growth cones following later on. Such early complex growth cones in other systems have been described as "pioneer" neurons (Bate, '76; Keshishian, '80; Ho and Goodman, '82; Bentley and Keshishian, '82; Yaginuma et al., '91). At the following stage in the wallaby, signalled by the elaboration of terminal arborizations, the growth cones distributed over the whole SC collapsed and became slender and rod-like. Although there is no direct evidence in the present study showing that the collapse was caused by an interaction with postsynaptic cells, evidence that particular types of neurites can induce collapse of growth cones has been obtained widely from *in vitro* studies. In chick, interactions between individual growth cones and

neurites from a variety of neuronal sources *in vitro* indicated that growth cones, encountering specific neurites, often undergo a morphological collapse (Kapfhammer and Raper, '87a.b). Studies of retinotectal connections in the tectum (Cox et al., '90; Stahl et al., '90) and of motor axon outgrowth (Norris et al., '89; Davies et al., '90) also provided evidence, *in vitro*, of growth cones collapse accompanied by guiding membrane-associated molecular cues. As well, later study in frogs suggested that collapse of growth cone is in part due to the different cell surface charateristics of tectal cells (Johnston and Gooday, '91). As in the wallaby, morphological changes in growth cones at different developmental stages were also observed in the cat (Ghosh and Shatz, '92). Like the wallaby growth cones, growth cones of LGN axons were more complex early in development. More complex growth cones with elaborate filopodial forms were also found in mouse (Bovolenta and Mason, '87), as they came to their target region, LGN during early development.

Impulse activity and the patterning of retinocollicular connection. In mammals (for reviews see Fawcett and O'Leary, '85; Udin and Fawcett, '88; Constantine-Paton et al., '90; Shatz, '90a,b), there are two well studied projections in which the role of impulse activity may be important in refining connections: the projection from each eye to the lateral geniculate nucleus (LGN) and the projection, in turn, from the LGN to layer 4 of the primary visual cortex. Neither the eye-specific layers within the LGN nor the ocular dominance columns within the cortex are present initially. However, activity-dependent competitive interactions from the two eyes can account for the establishment of these highly segregated sets of connections during development (Sretavan and Shatz, '86; Miller and Stryker, '90). The demonstration that this is the case was confirmed by experiments in which the inputs from both eyes were completely silenced

by injecting tetrodotoxin (TTX), a sodium channel blocker, postnatally (Stryker and Harris, '86). Segregation of the LGN axons into patches in layer 4 of cortex was prevented completely, and neurons in layer 4, normally monocularly driven, were instead binocularly driven, reminiscent of the initial period of normal postnatal development. The hypothesis is presumed to operate even before the retina is fully developed, using only correlations in spontaneous activity among neighbouring retinal ganglion cells. Such spontaneous firing could supply activity-dependent cues, provided that ganglion cells firing in the two eyes was asynchronous. Activation of the N-methyl-D-aspartate (NMDA) receptor is postulated to be a mechanism to initiate maintenance of simultaneously active synapses. Involvement of the NMDA receptor in mammalian visual development demonstrated a disruption of retinogeniculate afferent segregation by antagonists to NMDA receptors, causing the LGN cells to markedly reduce levels of activity (Hahm et al., '91). Involvement of the NMDA receptor also demonstrated that blockade of NMDA receptors prevents ocularity changes in mammalian visual cortex (Kleinschmidt et al., '87; Gu et al., '89; Bear et al., '90).

Evidence that impulse activity plays a role in the formation of topographic maps is less convincing. A developmental role for NMDA receptors was found in the initial establishment of topographic order in the retinocollicular projection of the rat by showing that blockade of NMDA receptor function interferes with the elimination of aberrantly positioned retinal ganglion cell arbors in the SC (Simon et al., '92). But this blockade of NMDA receptors did not totally disrupt the establishment of a topographic map in the SC. The development of correctly positioned arbors was not affected and the majority of aberrantly positioned arbors were still removed. This is perhaps not surprising as the major part of the

development of correctly positioned terminal arbors has occurred, before numerous spontaneous discharges and clear responses to optic nerve stimulation can be recorded (Molotchnikoff and Itaya, '93). Thus, evidence that activity plays a major role in the formation of terminal connections in their retinotopically correct position in the rat is poor because no electrical activity, spontaneous or evoked potential was obtained prior to the stage in which basic topography was established.

It seems clear in the wallaby that activity does not play a role in the initial formation of terminal zones in their retinotopically correct position because spontaneous activity or evoked potentials cannot be recorded prior to this stage (Freeman, '92). Over the second developmental stage, the size of terminal zones reduced with age and became more focused to a localized region. The synaptically evoked activity in neurons of the SC from stimulation of the optic nerve was recorded at this time. The possibility exists that this refinement may involve the pattern of electrical activity in the axons themselves.

Evidence for mammalian ties between marsupial mammals and placental mammals

In comparison with the present results obtained from the wallaby, a similar sequence of events and degree of precision in the developing retinocollicular pathway was also demonstrated by using a retrograde tracing method in cat (Ostrach et al., '86) and by anterograde tracing methods in cat and ferret (Snider and Chalupa, '93). Retrogradely labelled ganglion cells, after a focal collicular injection of tracer in the prenatal cat, were mainly concentrated in the topographically appropriate region of retina. When localized implants or an injection of DiI in the retina of fetal

animals was used anterogradely to label optic axons, a well-defined terminal zone was observed in both the contralateral and ipsilateral SC in the topographically appropriate region soon after the first contingent of fibres was found entering the SC, with some fibres extending outside the terminal zone followed later by the loss of most aberrant fibres. The morphological stages in the formation of eye specific laminae in the LGN of cat (Sretavan and Shatz, '84) also appeared similar to the formation of the retinocollicular projection in the wallaby. Individual retinogeniculate axons did not arborize widely in the LGN but took relatively straight paths and made short side branches, followed later by the elaboration of a single extensive axonal arbor within appropriate LGN territory accompanied by retraction of only a limited number of minor branches.

Two distinct growth modes of retinal projections to the SC were seen in Golgi studies, staining single axons and cells in hamster (Schneider et al. '85). In the first stage, in elongation mode, ganglion cell axons grew from the eye to the caudal end of the SC and they were unbranched except for tiny filopodial extensions, the characteristic of actively growing neurites. In the second stage, axons began to show the arborization mode, developing multiple collateral branches in the SC. A terminal arbor was elaborated from one of the branches, with others being gradually eliminated. Moreover, these two stages of growth of retinofugal axons were confirmed, by using three morphological methods: the Cajal-deCastro reduced silver method, the rapid Golgi technique and anterograde transport of HRP in hamster (Jhaveri et al., '91). Stages in the formation of retinal projections were also demonstrated in hamster using DiI as an anterograde tracer (Bhide and Frost, '91). First, axons of retinal ganglion cells elongated to their targets including the LGN and the SC. Then they simultaneously emitted unbranched or poorly branched collaterals to these

targets. In this study, the collateralization phase was regarded as a separate stage prior to axon arborization. Finally, the axons elaborated terminal arbors in their definitive targets in the LGN and the SC and eliminated their other collaterals. A similar pattern of the growth and arborization of retinocollicular axons was also obtained in mouse by means of filling of axons with HRP during postnatal development (Sachs et al., '86). Axons grew towards the caudal pole initially, extending primarily short and unbranched collateral sprouts. This was followed by the elimination of all but one or two collaterals which branched to form a single focal arbor. It was assumed that the focal arbors formed in retinotopically appropriate positions as, unlike in the wallaby, the retinal origin of axons was not known. In the above studies, a delay was observed between the arrival of optic axons in their targets and initiation of terminal formation within target fields. Similarly, the developing retinocollicular projection in the marsupial wallaby underwent two distinct growth patterns. There was initially an elongation of retinal axons in coarse topographical order, followed by terminal axonal arborization in their retinotopically correct locations with the loss of the initially widely distributed axons. Although quantitative data for the number of collaterals or branches formed during development in hamster and mouse is not available, it appeared less common in the wallaby, since only 9.3-12.6% of labelled axons were found to have side branches during development. A delay between axon arrival at targets and onset of arborization was also demonstrated in the wallaby, which could be a reflection of axons waiting for a maturational change to occur in the retina or in the SC.

However, retinocollicular axons in both albino and pigmented rat appear to go through a stage during the development of the projection (Simon and O'Leary, '90, '91, '92a,b,c), which is different from that seen in the wallaby.

Early in development, the retinal axons extended and branched widely over most of the SC, well beyond the location of their correct terminal zone. Axons typically formed side branches and often arborized at topographically incorrect positions throughout the SC, although they appeared to branch preferentially in a region, including but considerably larger than the correct terminal zone. 78% of all labelled peripheral nasal axons before the formation of their correct terminal zones, had at least one branch in the inappropriate rostral half of the SC. This had dropped to 24% at the time of the formation of focal terminals. In contrast, in the wallaby, only 11.6% of nasal axons formed side branches in ether the rostral or the caudal half of the SC prior to appearance of terminal zones. Similar number of branches were seen at the time of the formation of terminal zones. Unlike the wallaby where terminal zones only formed at their retinotopically correct positions, the achievement of precision of the neuronal connections in the rat included the elimination of many axons branches and topographically aberrant arborizations over time.

Retrograde tracing studies also demonstrated the difference in the development of retinocollicular projections between rat and wallaby. With injections of the retrograde tracer fast blue into caudal SC of neonatal albino rats, as well as a high density of labelled ganglion cells in the periphery of nasal retina, a lower density was also labelled in the remote temporal periphery, estimated to amount to 14% of the number labelled nasally (O'Leary et al., '86). In pigmented rat, Yhip and Kirby ('90) also reported that there were a small group of ganglion cells scattered erroneously in peripheral retina remote from those positioned appropriately. Instead, the results from retrograde tracing in wallaby (Marotte,'93) showed that, during development, the location of the majority of labelled retinal ganglion cells was observed to be in appropriate

topographic register with the deposit site in the SC, while the inappropriately positioned labelled ganglion cells were detected between the densely labelled patch and those in the central retina and both dorsal and ventral, adjacent to the patch rather than remote from the position of appropriately positioned cells. The retinocollicular pathway in the wallaby, as well as in the cat, ferret, hamster and mouse appears to be organized more accurately during development than that seen in the rat.

Comparison with the development of the retinotectal projection in non-mammalian vertebrates

The developmental plan of the formation of retinotopy in the non-mammalian vertebrates is different in some ways from that observed in the wallaby, in which retinal axons grow into the SC and are distributed in rough topographic order with no functional connections between retinal axons and collicular cells, followed much later by the formation of terminal axonal arborizations in their retinotopically appropriate locations and the functional synaptic connection. The first stage in the retinocollicular projection of the mammals is not seen in non-mammals. During normal development, the retinotectal projection of frogs and fish is topographically organized and functional from its inception (Chung et al., '74; Gaze et al., '74; Holt and Harris, '83; Holt, '84; Sakaguchi and Murphey, '85; O'Rourke and Fraser,'86, '90; Fujisawa, '87; Stuermer, '88a; Stuermer and Raymond, '89; Stuermer, '90; Kaethner and Stuermer, '92). In frogs the topographic ordering of optic axons is present from the beginning of tectal innervation and topographically ordered visual responses are recorded in the tectum at the same early time (Chung et al., '74; Gaze et al., '74; Holt and Harris, '83). These results were supported by the work of other neurobiologists (Holt, '84; Sakaguchi and Murphey, '85; Fujisawa, '87). An exception is that

at very early stages, nasal and temporal retinal arbors cover an overlapping tectal area transiently, with nasal arbors growing caudally along the rostrocaudal axis once caudal tectum is generated (O'Rourke and Fraser, '86, '90). In addition, results in fish showed that the retinotopic map is both retinotopic and relatively precise as soon as the axons occupy the tectal neuropil (Stuermer, '88a, '90). These axons were detected to run in straight routes to their target sites and form small terminal arbors exclusively confined to their retinotopically appropriate domains (Stuermer,'88a; Stuermer and Raymond, '89; Stuermer, '90). The visualization of the dynamics of retinal axon growth and arbor formation *in vivo* (Kaethner and Stuermer, '92) also revealed that retinotectal axons appear to grow in a directed manner and to arborize only at their retinotopically correct target sites.

The findings from the non-mammalian vertebrates implies that the retinal axons are highly selective in their target approach and choice from the time of ingrowth into the tectum. To maintain this organization, the projection undergoes continuous shifting of connections between retinal and tectal nuclei during development because of disparate modes of growth between retina and tectum (Gaze et al., '74, '79; Fraser, '83; Reh and Constantine-Paton, '84; Easter and Stuermer, '84; Fraser and Hunt, '86; Fujisawa, '87). Optic terminal arbors gradually shift in a caudomedial direction to allow fibres from newly added retinal cells to form connections along the rostrolateral edge of the tectum. The immediate onset of function which necessitates shifting of connections between ganglion cells and tectal nuclei found in non-mammals is presumably because fish and frog need to see immediately as they are free swimming larvae.

Other studies in non-mammalian vertebrates such as chicks also demonstrated different events in the development of the retinotectal projection from that seen in the wallaby. An investigation, on the basis of filling an eye with HRP following a small retinal lesion, indicated that chick retinal axons initially projected diffusely to the tectum and that topographically aberrant projections were eliminated during subsequent development (McLoon, '82). This pattern of the growth and arborization of chicken retinal ganglion cell axons was confirmed in more detail by labelling a small population of retinal axons with DiI (Nakamura and O'Leary, '89). During development, the growth and arborization of temporal retinal axons within the optic tectum of chicks was initially imprecise, with widespread development of axon branches and arbors including at inappropriate locations. However such axons did not extend into the caudal tectum. In this respect they are similar in behaviour to temporal axons in the wallaby. Subsequently, aberrant arbors, axons and axon segments that failed to form arbors in the appropriate terminal zone, were rapidly eliminated. Thus course correction and large-scale axonal remodelling led to the retinotopic ordering of terminal arborizations characteristic of the mature projection. In contrast, an investigation using an intraaxonally transported fluorescent marker by Thanos and Bonhoeffer ('87) showed that prior to the beginning of terminal arborization, the majority of axons arrive at their projection areas via straight, direct routes, and only a minority (<5%) of axons undergo a correction of their dorsal and ventral position, compared with about 40% of temporal axons doing so in Nakamura and O'Leary's study ('89). The latter suggested that regional differences in targeting errors may account for this dicrepancy. The suggestion that chick retinotectal connections like those in fish and frogs also shift during development has been made by McLoon ('85). This study showed that retinal axons form synaptic connections almost as soon as

they arrive in the tectum. The first connections appeared to be in the area where the axons enter the tectum, the rostroventral tectum, and they were formed by axons from ganglion cells in the central retina. In the mature projection, these cells form connections in the caudal tectum.

SUMMARY

The retinocollicular projection has been investigated anatomically to determine the establishment of the visual map in the developing wallaby. Two clear stages are demonstrated as a protracted period when retinal axons grow into the SC in coarse retinotopic order followed much later by a period when the terminal abrorizations are formed in the retinotopically appopriate position with the loss of more widely distributed axons. This notion of atwo stage process in the development of the retinocollicular projection is supported by a concomitant physiological experiment (Freeman, '92; Mark et al., 93), showing no recordable electrical activity during the first stage and the onset of evoked potentials to optic nerve stimulation during the second stage. Whether the organization of retinal axons in the optic nerve contributes to the retinotopic ordering in the SC is the aim in the next study.

Chapter 5. Retinotopic Organization In The Optic Nerve During Development

INTRODUCTION

One hypothesis used to explain the mechanisms controlling the formation of retinotopic connections of the retinocollicular projection is pre-ordering of retinal axons in the optic pathway (Horder and Martin, '78; Willshaw and von der Malsburg, '79; Bodick and Levinthal, '80; Bunt and Horder, '83; Udin and Fawcett, '88). "Pre-ordering" refers to a mechanism whereby retinal axons, as they grow, remain neighbours with axons that come from neighbouring neurons in the presynaptic structure, and therefore arrive at the postsynaptic structure already topographically arranged. That is, axons leave the retina in a topographic order and then establish a retinotopic organization in their primary target, the SC, by simply maintaining neighbour relationships throughout the optic pathway. Many experiments on the retinotopy in the optic nerve have been carried out in non-mammals and mammals. Retinal axons show order in the optic nerve but it is rather variable according to different species.

A variable degree of retinotopy of optic nerve axons has been reported in frogs. Only a rather imprecise organization of retinal axons in the optic nerve was observed in the investigations in adult *Xenopus* (Fawcett, '76, '81; Holt, '84) and adult *Rana pipiens* (Scalia and Arango, '83). Fibre bundles arising from coherent retinal ganglion cells do not stay coherent throughout the optic nerve. On the other hand, a precise order of retinal axons was reported in adult and developing *Xenopus* (Cima and Grant, '82; Bunt and Horder, '83; Taylor, '87) and *Rana* (Bunt and Horder, '83; Reh et

al., '83). Retinal axons were reported to be arranged on the basis of their age, that is, with fibres grouped together in chronotopic order (Cima and Grant, '82; Taylor, '87), or arranged topographically in which the organization of the optic nerve fibres reflected the retinal position of the ganglion cells that give rise to them (Bunt and Horder, '83; Reh et al., '83). Thus, the two different findings on the degree of organization of optic nerve fibres provided contrasting hypotheses to explain the formation of the visual map in the frog tectum, in which initial targeting of retinal axons in the developing tectum is organized topographically and an almost mature terminal arborization is ordered precisely as soon as the beginning of tectal innervation (for review see chapter 4). One seems to falsify the hypothesis that axonal ordering in the optic nerve passively maintained from the retina is sufficient for the precise retinotopic projection in the primary target, the tectum, by showing that the optic nerve was organized rather imprecisely (Fawcett, '76, '81; Scalia and Arango, '83). Instead, a different hypothesis was made from the other studies (Cima and Grant, '82; Bunt and Horder, '83; Reh et al., '83) proposing that the maintenance of retinal axons in specific spatial relationships throughout the optic nerve plays a role in the formation of ordered connectivity in the primary visual centre.

A high degree of order in the arrangement of fibres in the optic nerve, the first part of the visual pathway, was demonstrated in other nonmammalian vertebrates such as fish and chick. That retinal axons retained either retinotopic or chronotopic neighbourhood relationships throughout the optic nerve, was also demonstrated in adult fish. Precise order in the optic nerve was seen in adult cichlid fishes with ribbon-shaped optic nerves (Scholes, '79). The orderly layout of visual fibres in the optic nerve suggested that they keep the same neighbours throughout the trajectory of the optic nerve. Fibres also were found to be arranged with

fibres of the same age together in the nerve in so-called "chronotopic" order. This aspect of fish optic nerve organization was emphasized by experiments on adult goldfish (Rusoff and Easter, '80), in which the optic nerve was found to be composed of clusters of axons from ganglion cells born at the same time, and within each cluster neighbouring axons arose from neighbouring ganglion cells. Investigation with a double-label technique (Bunt, '82) showed a retinotopic and temporal organization in the optic nerve of adult goldfish. Fibres were found to form an orderly temporal sequence in the optic nerve, with the oldest fibres from the central retina on one side of the nerve and the youngest from peripheral retina on the other. As well, fibres from different positions around the circumference of the retina were also seen to project topographically to separate parts of the optic nerve, with fibres from the ventral retina on each edge of the nerve, the dorsal fibres in the centre, and the nasal and temporal fibres in between. This arrangement continued with only a little loss of precision up to the optic tract. More evidence for a retinotopic arrangement of optic axons throughout their transit of the optic nerve was also obtained in teleosts (Bunt and Horder, '83). However, a slightly different result showing a dispersion of ordering with increasing distance from the optic nerve head to the chiasm was demonstrated from adult goldfish (Easter et al., '81). As in adult fish, experiments reported in zebrafish embryo (Bodick and Levinthal, '80; Stuermer, '88a) also demonstrated a high degree of optic axon ordering in the developing optic nerve, where growing optic nerve fibres follow neighbours during embryogenesis. Like in fish, a relatively high degree of fibre order in the optic nerve was shown in adult and developing chick (Rager and Rager, '78; Rager, '80b; Thanos and Bonhoeffer, '83). Retinal axons from neighbouring ganglion cells cluster together as they travel in the optic nerve with either chronotopic or retinotopic organization. More evidence for an orderly

arrangement of optic axons within the optic nerve was also obtained from studies on different adult non-mammalian vertebrates including amphibia, teleosts, aves and reptilia (Bunt and Horder, '83; Reh et al., '83). In all these species it was shown that retinal axons maintain neighbourhood relationships throughout their pathway of the optic nerve. The precise arrangement of retinal axons in the optic nerve obtained from most studies in fish and chick and the fact that optic fibres project topographically onto the tectum in the same species (for review see chapter 4) led to the proposal that maintenance of retinotopic ordering throughout the optic nerve might be alone sufficient to account for the formation of a retinotopically ordered projection in the central target (Horder and Martin, '78; Scholes, '79; Bodick and Leventhal, '80; Rager, '80a,b; Bunt, '82; Bunt and Horder, '83).

It has been concluded from studies on some non-mammalian vertebrates that sequential differentiation of nerve cells could be translated into a well-defined spatial organization by simple mechanical principles governing the way in which the growing axons follow their substrate. However, this event seems to be unlikely to explain the two stages processes in establishment of ordering retinocollicular projection in the wallaby.

In the studies on mammalian vertebrates, a coarse retinotopy in the optic nerve and a major rearrangement, of an inversion of axons arising from dorsal and ventral retina, was found in both adult and developing quokka wallaby (Chelvanavagam and Beazley, '94) and adult rat (Bunt and Lund, '82; Chelvanavagam and Beazley, '92). The study in rat also suggested that some order is lost as axons travel down the optic nerve (Bunt and Lund, '82; Chelvanavagam and Beazley, '92). However, there was only a very limited degree of retinotopic order with the retinal axons dispersing largely

along the length of the optic nerve, in the developing optic nerve of albino rat (Simon and O'Leary, '91). Furthermore, this loose retinotopic arrangement of retinal axons with great scatter along the optic nerve was also observed from studies on cat and monkey. It was apparent in the cat optic nerve (Horton et al., '79; Naito, '86), that beyond the optic nerve head no strict retinotopy is present and axons of adjacent retinal ganglion cells do not generally travel side by side in the nerve although some crude topography may be preserved. The optic nerve axons showed a tendency to scatter toward the chiasm such that it is unlikely that they maintain their initial fibre topography along the entire length of the optic nerve. As well, in monkey (Naito, '89), only a crude topographic order was reported in the optic nerve, with a great deal of dispersion of fibres from any particular part of the retina. Moreover, in embryonic monkey, individual retinal ganglion cell axons were not observed to retain a stable group of neighbouring fibres as they grew through the optic nerve during development (Williams and Rakic, '85). Thus, in the mammalian vertebrates, less degree of organization of retinal ganglion cell axons exists in the optic nerve at all stages from early development to adult when the retinocollicular terminal fields are well organized (Lund, '78).

From the studies on the development of retinotopy in the SC (chapter 4), in the first stage in the development of the retinocollicular projection prior to the formation of terminal arborizations in the topographically appropriate zones, developing retinal axons are distributed initially in a coarse order. Whether the mechanism of preordering of retinal axons in the optic pathway, which has been suggested to be sufficient to generate the topographic order of retinal termination in the developing tectum in non-mammals, plays a role in the formation of retinocollicular projections in mammals such as wallaby became a focus in the present study. The

approach which can be used to identify the possible mechanisms underlying the retinotopic organization in the SC during early development, is to look at whether the retinal axons maintain topographical neighbour relationships in the optic nerve initially and whether the axons in the optic nerve undergo a refinement in accordance with that in the target later on. That is, whether the correlation between axon organization in the optic nerve and the establishment of the retinotopic map in the SC exists in the developing wallaby.

The degree of retinotopy in the optic nerve was studied at different stages of development. The same methods detailed in chapter 4 were used. The fluorescent anterograde axonal tracer, DiI was placed in the periphery of different retinal quadrants to label retinal ganglion cell axons in the optic nerve. To match with the development of the retinocollicular projection, pouch young wallabies at four developmental stages aged 27-28, 41-47, 61-68 and 90-95 days were used. At 27-28 days, retinal axons project to the SC in a coarse retinotopic order. From 41-47 days, terminal arborizations begin to form in the retinotopically appropriate regions in the SC. By 61-68 days terminal zones are defined clearly with the disappearance of the initially more widely distributed axons. From 91-95 days, discrete terminal zones are present. Retinotopic order in the developing optic nerve and the order in the context of the formation of the visual map in the SC will be described.

MATERIALS AND METHODS

Animals:

Thirty eight pouch young wallabies *(Macropus eugenii)* ranging from 27-28 (n=10), 41-47 (n=10), 61-68 (n=10) and 90-95 (n=8) days were used. The age of all animals was determined from their birth date or from a chart of head lengths of animals of known age (W.E. Poole, personal communication). The retina with DiI deposits and the SC in these animals had been observed in the previous study (chapter 4).

Labelling axons

Labelling methods for these animals has been described in chapter 4 (see p76 for detail). Briefly, the animal was anaesthetised by either hypothermia or by intramuscular ketamine and xylazine. A small piece of gelfoam impregnated with DiI was inserted into the retina through the sclera. Localized placement of DiI was made in each of four different regions of the retina according to the pigment line which crosses the temporonasal pole.

Histology

The histological procedures used after making DiI deposits are described in chapter 4 (see p77 for detail). Wholemounts of retina were made and the location of fluorescent labelling viewed in a Leitz fluorescence microscope. The entire optic nerve was dissected from the level behind the optic nerve head to the level just prior to the chiasm. The position of the pigment line in the retina, which appeared from 28 days onwards was also used to

determine the orientation of the optic nerve. The orientation of the optic nerve was marked dorsally by a suture in the nerve sheath. The tissue was then embedded in gelatin/albumin and blocked (chapter 2, see p26 for detail). Sections were cut transversely at 150 to 200 μm in thickness on a vibratome. Every section was mounted serially on glass slides with 0.1 M phosphate buffer.

Analysis

The sections of optic nerve were examined and photographed under DiI fluorescence illumination with the excitation filter, M2, Bp546/14. The outline of the cross section of the optic nerve, the position and the density of labelled axons were then reconstructed from the photos. In all but one case labelling from five levels along the optic nerve was traced: adjacent to the eye, just prior to the chiasm, and at three equally spaced intervals in between. Either the position of the labelled region or individual axons were marked, depending on the age of the animals. Prior to 90 days, not every individual axon was marked as axons were present in high density and individual axons were not easily resolvable at a light microscope level or there were large numbers of overlapping axons labelled by DiI. However, an impression of the density of labelled axons is given.

By means of an IBM compatible computer imaging system with the scientific measurement program, Sigma-Scan version 3.0 (Jandel Scientific), the area of the optic nerve and the area covered by labelled axons was measured at three levels: a point adjacent to the eye, midway along optic nerve, and a point just before the chiasm. The percentage area covered by labelled axons in the optic cross sections was calculated.

RESULTS

All the figures for this chapter are grouped together at the end of the results section.

DiI labelling in the retina

Focal deposits of DiI were made into the periphery of each of four retinal quadrants of animals aged 27-28, 41-47, 61-68 and 90-95 days. A typical DiI placement is shown in Fig.4.1 (chapter 4). The DiI deposits covered an average of 1.8% in retinal area, ranging from 0.5% to 6.7% (n=38, SD [standard deviation] =±1.3). In the retina, labelled axons travelled together in a small compact fascicle directly from the DiI deposit site to the optic disc. The labelled axons were clustered together as they passed through the optic disc. From 61-68 days, there were many animals in which no axons were followed between the DiI deposit site and the optic disc although the ganglion cell terminals were demonstrated in their target, the SC (chapter 4). In the present study, all labelled axons which left the eye were seen to originate from a single DiI placement site and no ectopic labelling sites were identified. Thus, the findings in this study are based on descriptions of retinal axons directly labelled from a small localized dye deposit.

Order and distribution of labelling in the optic nerve

The length of the optic nerve was estimated to be between 3 to 5 mm depending on age from 27 to 95 days. The nerve was cylindrical for most of its length but tended to become slightly flattened dorsoventrally as it reached the chiasm.

27-28 days (n=10)

At this stage, a rough retinotopy was observed in the optic nerve where labelled axons from each of the different regions of retina were grouped together in the nerve. The average area covered by labelled axons from different quadrants in the cross sections of the optic nerve was 47% and the retina covered by DiI deposits was 2.3%. Axons dispersed slightly as distance from the optic nerve head increased. A high density of labelled axons was found at this stage.

Temporal deposit (n=2) Axons from the temporal retina occupied the corresponding region of nerve adjacent to the optic nerve head. The labelled axons in the nerve were distributed much more widely than the DiI deposit in the retina. Away from the optic nerve head, axons dispersed slightly although the axons still maintained a group. The optic nerve axons strongly dominated the lateral and slightly dorsal side of the nerve (Fig.5.1). The border of the labelled region was sharp and labelling in the other half was not detected (Fig.5.2A).

Nasal deposit (n=3) Labelled axons from the nasal retina were clustered on the medial side of the optic nerve, the corresponding position of nasal retina behind the optic nerve head. The axons then slightly spread out to occupy a larger proportion of the optic nerve away from the optic nerve head (Fig.5.3). A high density of labelled axons were present within the labelled region on the medial side of the nerve (Fig.5.2B).

Dorsal deposit (n=2) The labelled axons from the dorsal retinal origin occupied the dorsal optic nerve for a short distance away from the optic nerve head. The axons then gradually spread from the dorsal side through

the middle of the nerve to the ventral side, along the course of the optic nerve. By midway along the length of the optic nerve, the axons had shifted to occupy a region in the ventral aspect of the optic nerve (Fig.5.4, 5.5).

Ventral deposit (n=3) Two patterns of axons from the ventral retina were found in the optic nerve. In two cases, ventral axons occupied a crescent shaped region ventrally close to the optic nerve head. Gradually, by splitting into two groups, the axons shifted dorsally around the periphery of the nerve as distance from the optic nerve head increased. Prior to the chiasm, the labelled axons were distributed in a crescent-shaped region of the dorsal nerve (Fig.5.6, 5.7). In another case, labelled axons from the ventral retina were distributed ventrally in a crescent-shaped region at one side of the nerve adjacent to the eye. Along the length of the optic nerve, the axons gradually moved dorsally, mainly around the lateral periphery of the nerve, with a few axons seen medially. Close to the chiasm, the labelled axons occupied a crescent shaped region dorsally and laterally as in the other two cases (Fig.5.8).

41-47 days (n=10)

By this stage, a small increase in the degree of retinotopic order was seen in the optic nerve, with the area covered by labelled axons reducing to 32% on average. The retina covered by DiI was an average of 1.4%.

Temporal deposit (n=5) In four cases, as axons labelled by DiI in the temporal retina entered the optic nerve, they occupied the corresponding position. The temporal axons then moved slightly dorsally as they ran along the optic nerve from the optic nerve head to the chiasm (Fig.5.9). The axons were confined clearly in one group (Fig.5.10A). In one case, temporal

axons were distributed around the periphery of the lateroventral nerve close to the eye and then divided into two groups, with the main group in the lateral nerve and another small group in the medial nerve. Gradually, the two groups of axons shifted dorsally around the periphery of the lateral and medial nerve and occupied a region temporodorsally prior to the chiasm (Fig.5.10B, 5.11).

Nasal deposit (n=2) Behind the optic nerve head, labelled axons from the nasal retina were distributed in the corresponding position of the optic nerve. The nasal axons spread out slightly and shifted to a medioventral position along the course of the optic nerve (Fig.5.12). The axons were clustered together in a relatively defined region (Fig.5.13A).

Dorsal deposit (n=1) In this case, close to the optic nerve head, labelled axons from the dorsal retina were positioned around the periphery of the nerve dorsally and slightly laterally. The axons shifted towards the opposite side by passing through the middle portion of the nerve. When the nerve ended at the chiasm, the labelled axons coming from the dorsal retina were distributed more diffusely, mainly in the ventral part of the optic nerve (Fig.5.13B, 5.14).

Ventral deposit (n=2) In both cases, axons from the ventral retina initially extended along the periphery of the ventral part of the optic nerve close to the optic nerve head. The labelled axons then shifted gradually to the opposite side of the nerve, mainly along the medial side of the nerve but with a few axons running laterally. Close to the chiasm, the axons were positioned dorsally in a crescent-shaped region of the optic nerve and were distributed slightly more widely than they were close to the optic nerve head (Fig.5.15, 5.16).

61-68 days (n=10)

At this age, the degree of order of labelled axons remained unchanged in the optic nerve. A similar area to that obtained in the previous age group was covered by labelled axons, with an average of 35% of the cross-section of the optic nerve. The area covered by DiI deposits in the retina was 0.9% on average. Density of labelled axons decreased and the individual axons were resolvable.

Temporal deposit (n=1) When labelled axons from the temporal retina entered the optic nerve, they were confined to the corresponding region on the lateral side of the optic nerve. Close to the chiasm, the labelled axons were distributed slightly more widely and had shifted to a temporodorsal position (Fig.5.17). Individually labelled axons could be seen within the labelled region (Fig.5.18A).

Nasal deposit (n=4) Close to the optic disc, labelled axons from the nasal retina were distributed on the corresponding medial side of the optic nerve. The axons gradually dispersed and shifted slightly ventrally along the optic nerve. Near the chiasm, the labelled axons were clustered medially and ventrally (Fig.5.18B, 5.19).

Dorsal deposit (n=3) Close to the optic nerve head, labelled axons from the dorsal retina were confined to the dorsal part of the nerve. The axons gradually migrated ventrally through the central region of the nerve. Close to the chiasm, they were scattered in a region in the ventral part of the nerve (Fig.5.20, 5.21).

Ventral deposit (n=2) In these cases, labelled axons from the ventral retina split into two groups a short distance away from the optic nerve head and shifted dorsally from the ventral nerve primarily around the periphery of the nerve. As the axons were traced to a location prior to the chiasm, they were distributed mainly around the periphery of the dorsal optic nerve (Fig.5.22, 5.23).

90-95 days (n=8)

At this age, the degree of topography of axons from different regions of the retina remained unchanged in the optic nerve. After DiI deposits with an average labelling of 1.9% in the retina, the average area covered by labelled axons was 35% in the optic nerve. A lower density of labelled axons was seen and the axons were distributed only sparsely in the optic nerve.

Temporal deposit (n=2) Close to the optic nerve head, labelled axons from the temporal retina were distributed sparsely at the corresponding lateral side of the optic nerve. The axons shifted slightly dorsally with increasing distance from the optic head towards the chiasm (Fig.5.24, 5.25).

Nasal deposit (n=2) As labelled axons from the nasal retina entered the optic nerve, they were spread sparsely over the corresponding medial side of the nerve. The axons moved slightly ventrally away from the optic nerve head, and close to the chiasm they were distributed medially and ventrally (Fig.5.26, 5.27).

Dorsal deposit (n=2) Close to the optic nerve head, axons labelled by DiI in the dorsal retina were distributed dorsally in the optic nerve. Along the course of the optic nerve, the axons then shifted gradually ventrally through the middle portion of the nerve. Close to the chiasm, the axons were localized ventrally in the nerve (Fig.5.28, 29A).

Ventral deposit (n=2) In the two cases, close to the optic nerve head, labelled axons from the ventral retina were distributed ventrally in a defined region in the nerve. The axons shifted gradually to the dorsal portion of the nerve along the medial periphery of the nerve. Close to the chiasm, the axons occupied a diffuse crescent-shaped area dorsally (Fig.5.29B, 5.30).

Quantitative data of DiI deposits in the retina and the distribution of labelled axons in the optic nerve

The size covered by the DiI deposit in the retina ranged from 0.9% to 2.3% on average in the different age groups from 27 to 95 days. The small variation of DiI deposit in the retina did not correlate with the changes of percentage of optic nerve occupied by labelled axons (Fig.5.31).

The percentage of optic nerve covered by labelled axons from different quadrants is shown in figure 5.31. In the optic nerve, temporal axons initially occupied an average of 43% of the cross-sectional area at 27-28 days. This area covered by labelled axons decreased to 25-26% from 41-47 days onwards. Dorsal and ventral axons showed similar changes in the percentage of the optic nerve they occupied, where the area covered by labelled axons decreased from 45% and 53% at 27-28 days to 34% and 31% at 41-47 days with little change at later stages. Nasal axons occupied 49%

of the nerve initially at 27-28 days, with no consistent change during development.

At each age group in the present study, axons dispersed to a small extent along the length of the optic nerve. The region covered by labelled axons in the optic nerve, pooled for all retinal quadrants, increased slightly as distance from the optic nerve head increased (Fig.5.32).

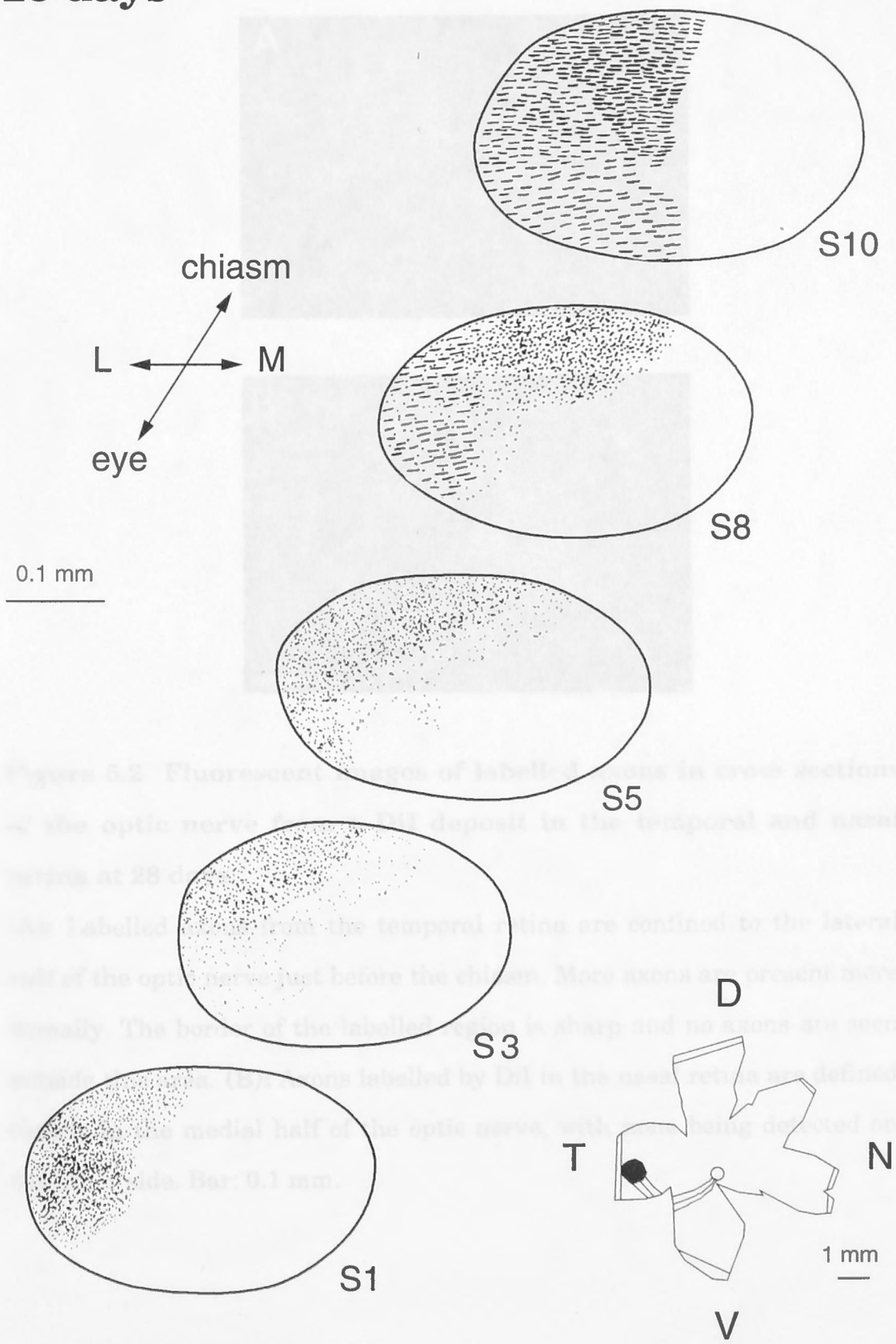
The area occupied by labelled axons in the optic nerve, pooled for each age, was not found to correlate with axon coverage in the SC in the same animals at the same age (chapter 4). The degree of retinotopic order in the nerve did not undergo any dramatic changes from 41 to 95 days since average percentage of area covered by labelled axons in the optic nerve remained unchanged at 32-35% after a drop from 47% at 27-28 days. However, over the same time there was a large refinement of the retinal projection in the SC. The initially more widely distributed axons in the SC reached a peak covering an average area of 37% at 41-47 days and they declined dramatically to occupy an average area of 6-7% as focal terminal zones were present at 61 to 95 days (Fig.5.33).

Figure 5.1 Line drawings of cross sections of the optic nerve showing the area covered by labelled axons in the nerve at 28 days

Left: Distribution of labelled axons in the optic nerve. Axons labelled by DiI in cross sections of the optic nerve are shown by dots and lines. At the point behind the optic head (S1), labelled axons from the temporal retina are distributed mainly in the lateral part of the nerve and they shift slightly dorsally (S3, S5, S8). The axons disperse slightly as distance from optic nerve head increases. At the level prior to the chiasm (S10), the lateral half of the optic nerve is occupied by labelled axons, and more axons are distributed in temporodorsal nerve. L: lateral; M: medial; S: section.

Right: Location of deposit of DiI in the retina. DiI deposit in temporal retina is in solid black. Open circle marks the optic disc (OD). Labelled axons can be seen between the deposit and the OD. The labelled region containing axons is outlined rather than every individual axon being drawn. T: temporal; N: nasal; D: dorsal; V: ventral.

28 days



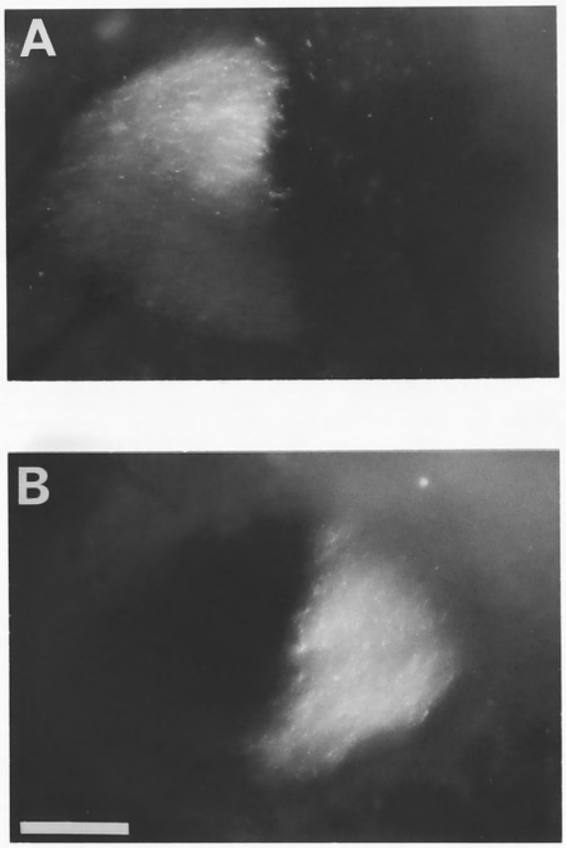


Figure 5.2 Fluorescent images of labelled axons in cross sections of the optic nerve from a DiI deposit in the temporal and nasal retina at 28 days

(A): Labelled axons from the temporal retina are confined to the lateral half of the optic nerve just before the chiasm. More axons are present more dorsally. The border of the labelled region is sharp and no axons are seen outside this area. **(B):** Axons labelled by DiI in the nasal retina are defined clearly in the medial half of the optic nerve, with none being detected on the other side. Bar: 0.1 mm.

Figure 5.3 Line drawings of cross sections of the optic nerve with a nasal DiI deposit in the retina at 28 days

Conventions are the same as for figure 5.1. In addition, the line crossing temporonasally in the retina is the pigment line which indicates the border of light (upper) and heavy (lower) pigmentation. Labelled axons from the nasal retina cover the medial side of the optic nerve and spread out slightly along the length of the optic nerve, with higher density in the centre of labelling (S2, S5, S7, S9, S12). DiI deposit is in nasal retina and slightly ventral.

28 days

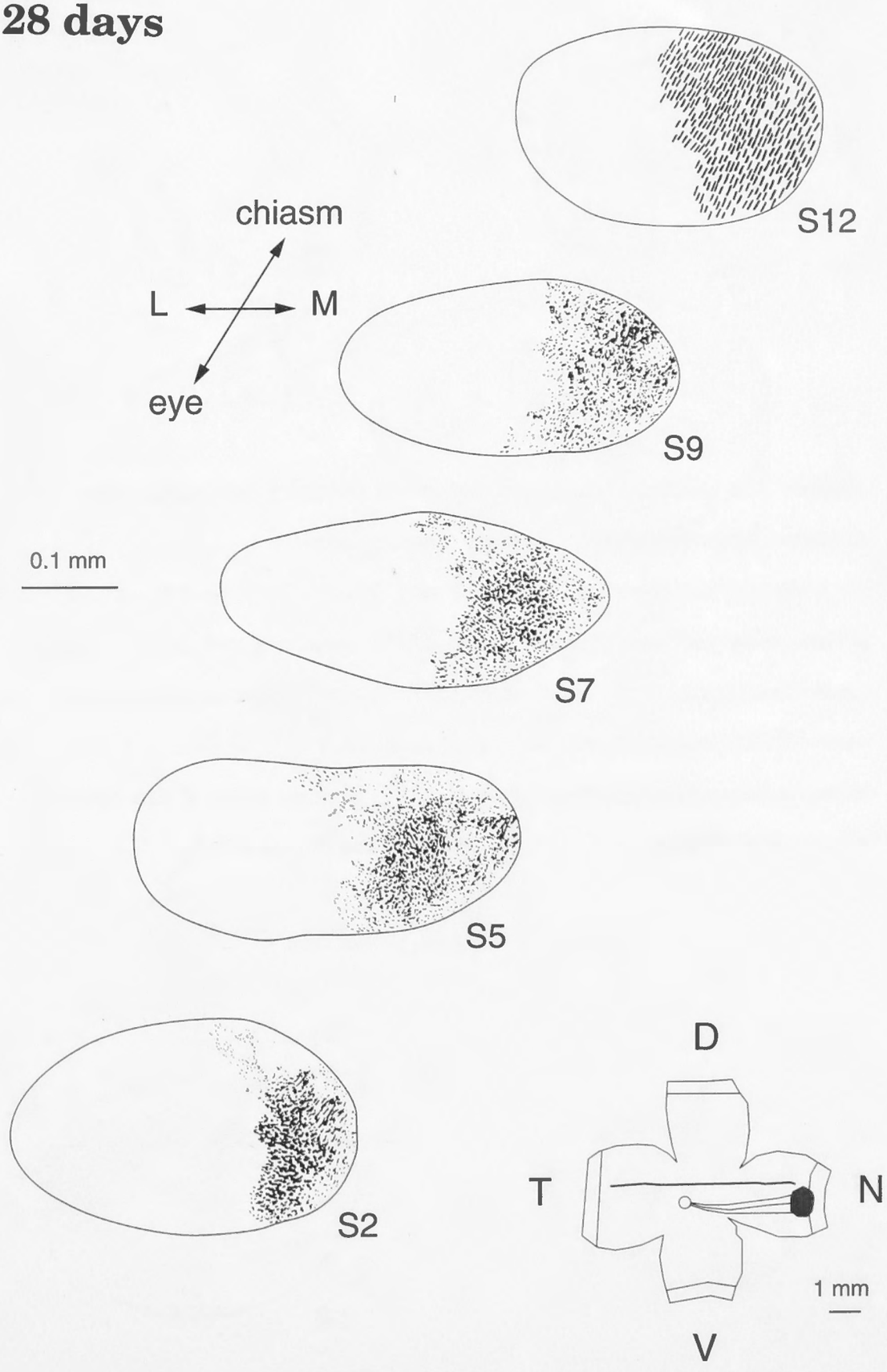
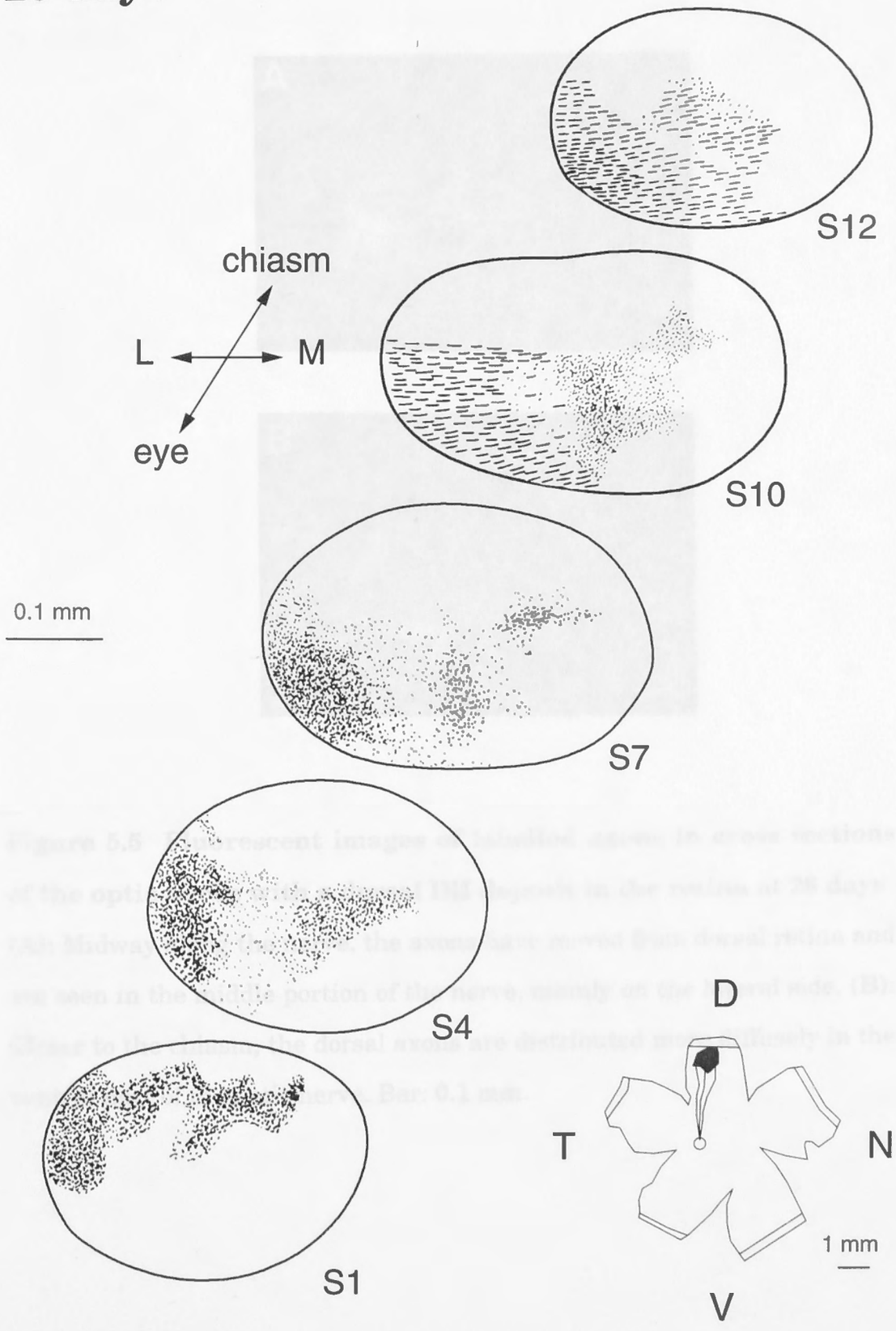


Figure 5.4 Line drawings of cross sections of the optic nerve with a dorsal DiI deposit in the retina at 28 days

Conventions are the same as for figure 5.1. Labelled axons from the dorsal retina enter the corresponding region of the optic nerve. A short distance away from optic nerve head, they gradually cross over to the opposite side ventrally through the central region (S1, S4, S7, S10). Finally, the dorsal axons are scattered more diffusely in the ventral part of the optic nerve close to the chiasm (S12). DiI deposit is in the dorsal retina.

28 days



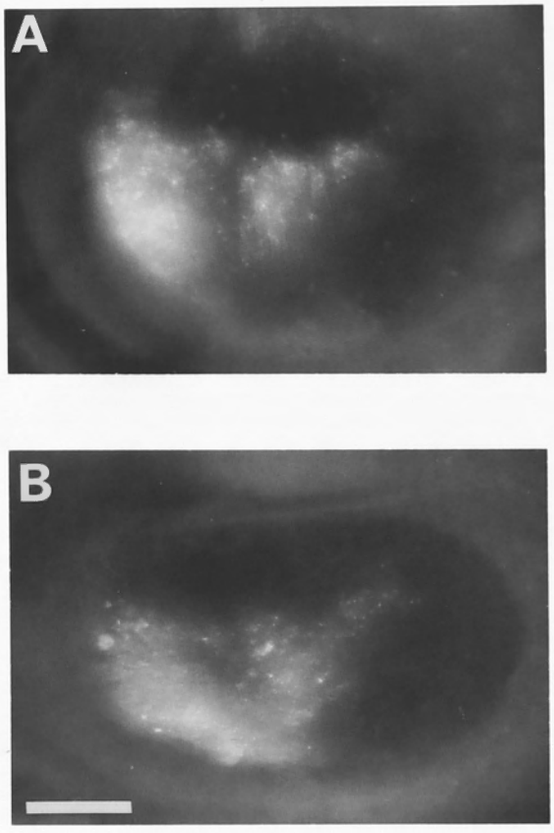


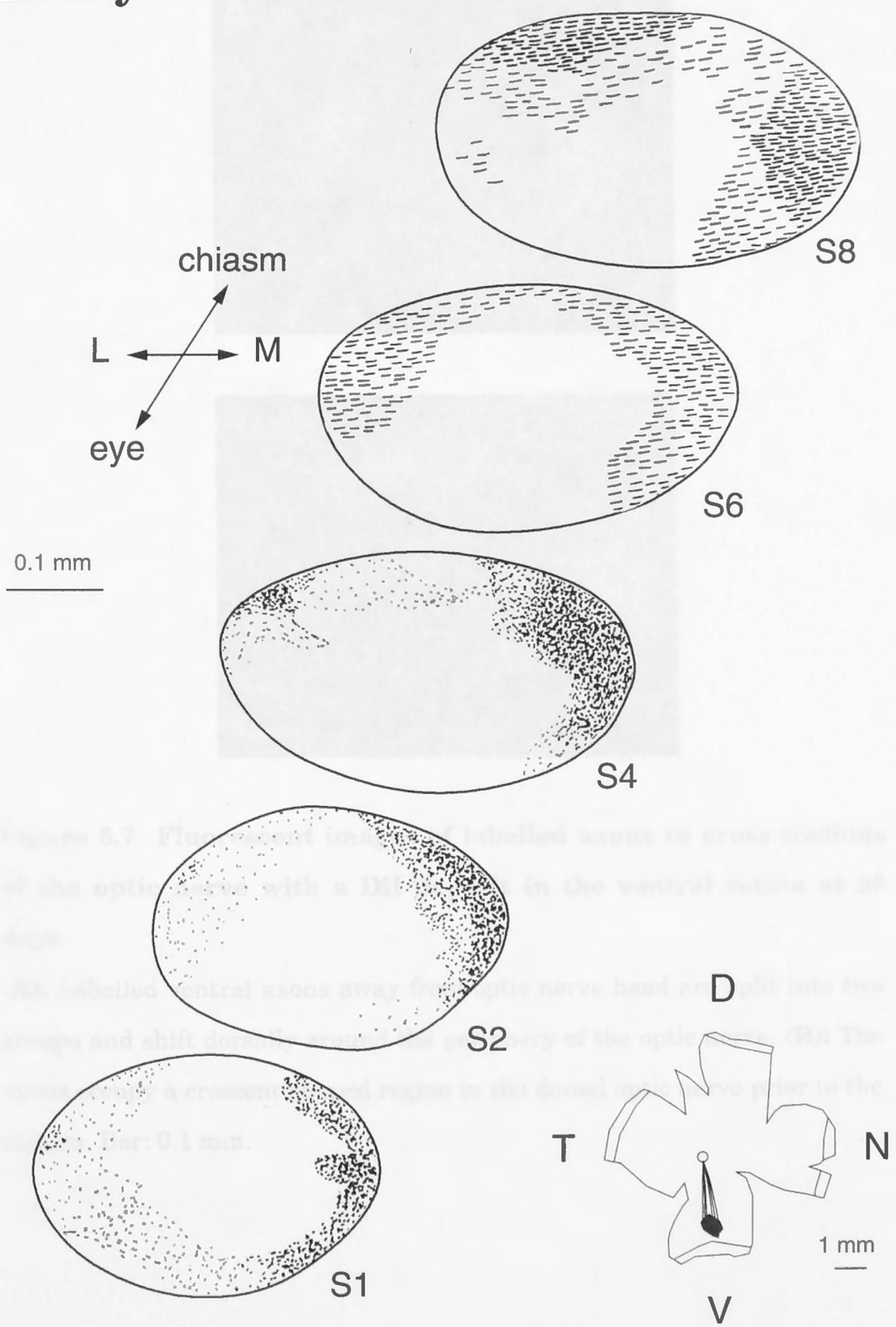
Figure 5.5 Fluorescent images of labelled axons in cross sections of the optic nerve with a dorsal DiI deposit in the retina at 28 days

(A): Midway along the nerve, the axons have moved from dorsal retina and are seen in the middle portion of the nerve, mainly on the lateral side. **(B)**: Closer to the chiasm, the dorsal axons are distributed more diffusely in the ventral part of the optic nerve. Bar: 0.1 mm.

Figure 5.6 Line drawings of cross sections of the optic nerve with a ventral DiI deposit in the retina at 28 days

Conventions are the same as for figure 5.1. One way of ordering of ventral axons seen in the optic nerve is shown here. Figure 5.8 shows the other pattern of ordering seen for ventral axons. The axons occupy a crescent shaped region ventrally close to the optic nerve (S1). Gradually, by splitting into two groups with more axons medially, the axons shift dorsally around the periphery of the nerve away from the optic nerve head (S2, S4, S6). Prior to the chiasm, the labelled axons are distributed more diffusely in a crescent shaped region of the dorsal nerve (S8). DiI deposit is in the ventral retina.

28 days



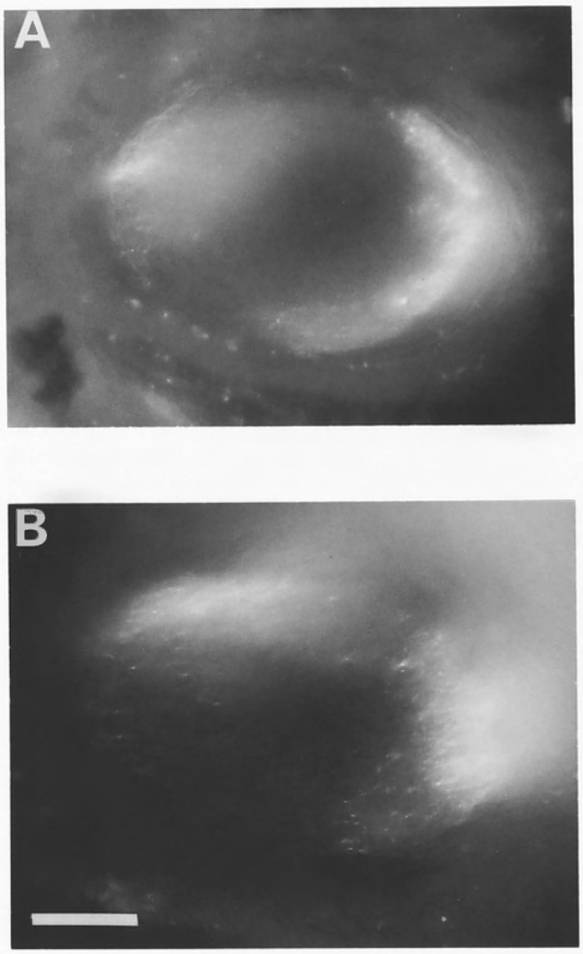


Figure 5.7 Fluorescent images of labelled axons in cross sections of the optic nerve with a DiI deposit in the ventral retina at 28 days

(A): Labelled ventral axons away from optic nerve head are split into two groups and shift dorsally around the periphery of the optic nerve. **(B):** The axons occupy a crescent shaped region in the dorsal optic nerve prior to the chiasm. Bar: 0.1 mm.

Figure 5.8 Line drawings of cross sections of the optic nerve with a ventral DiI deposit in the retina at 28 days

Conventions are the same as for figure 5.1. A different pattern of ordering of ventral axons is shown here. Ventral axons occupy a region at one side of the nerve ventrolaterally (S2) close to the optic nerve head and gradually shift dorsally, mainly around the lateral periphery of the nerve (S6, S10, S15). Close to the chiasm, the labelled axons occupy a crescent shaped region dorsally, with more axons laterally (S19). A focal deposit of DiI is in the ventral retina.

28 days

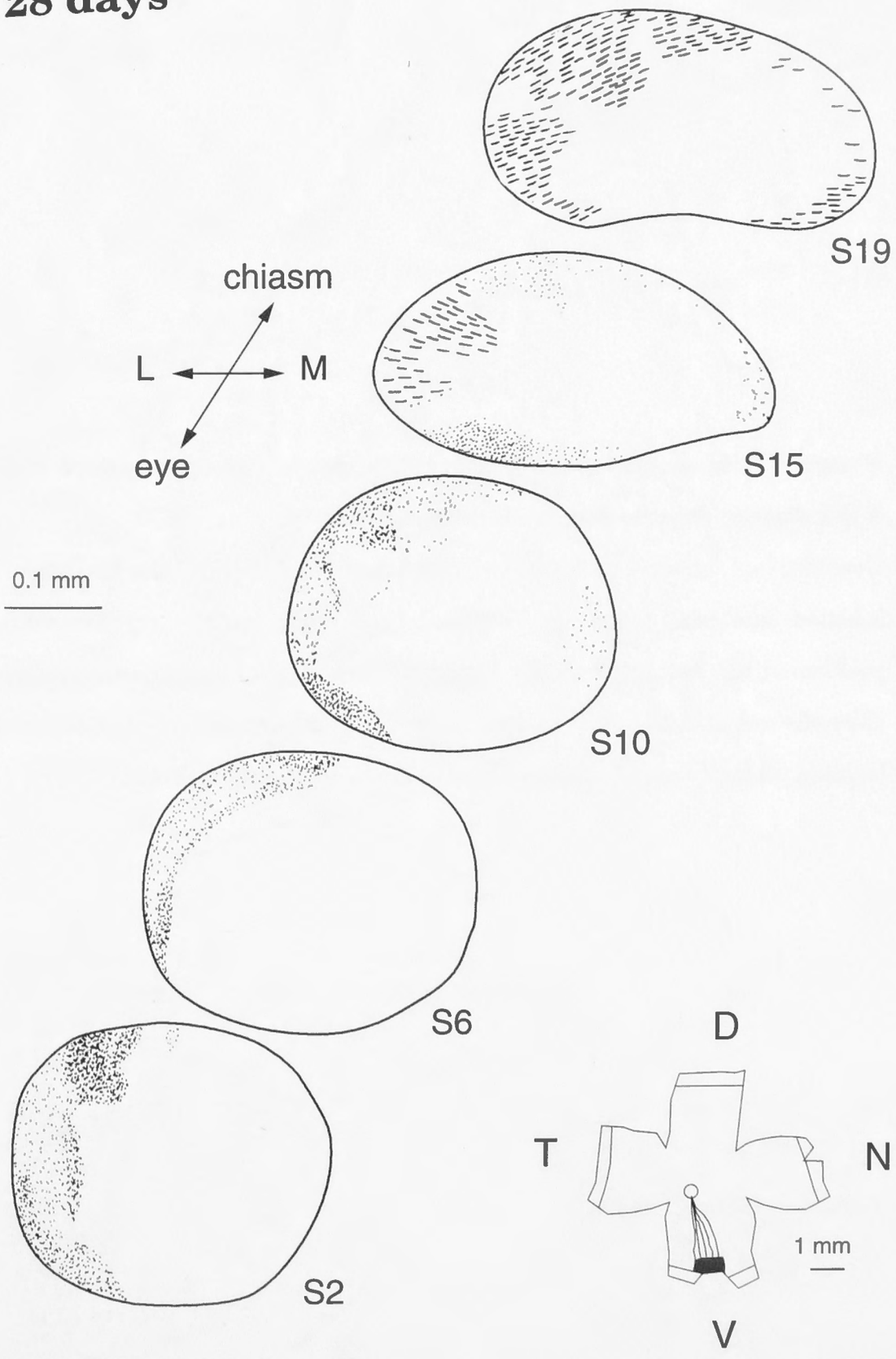
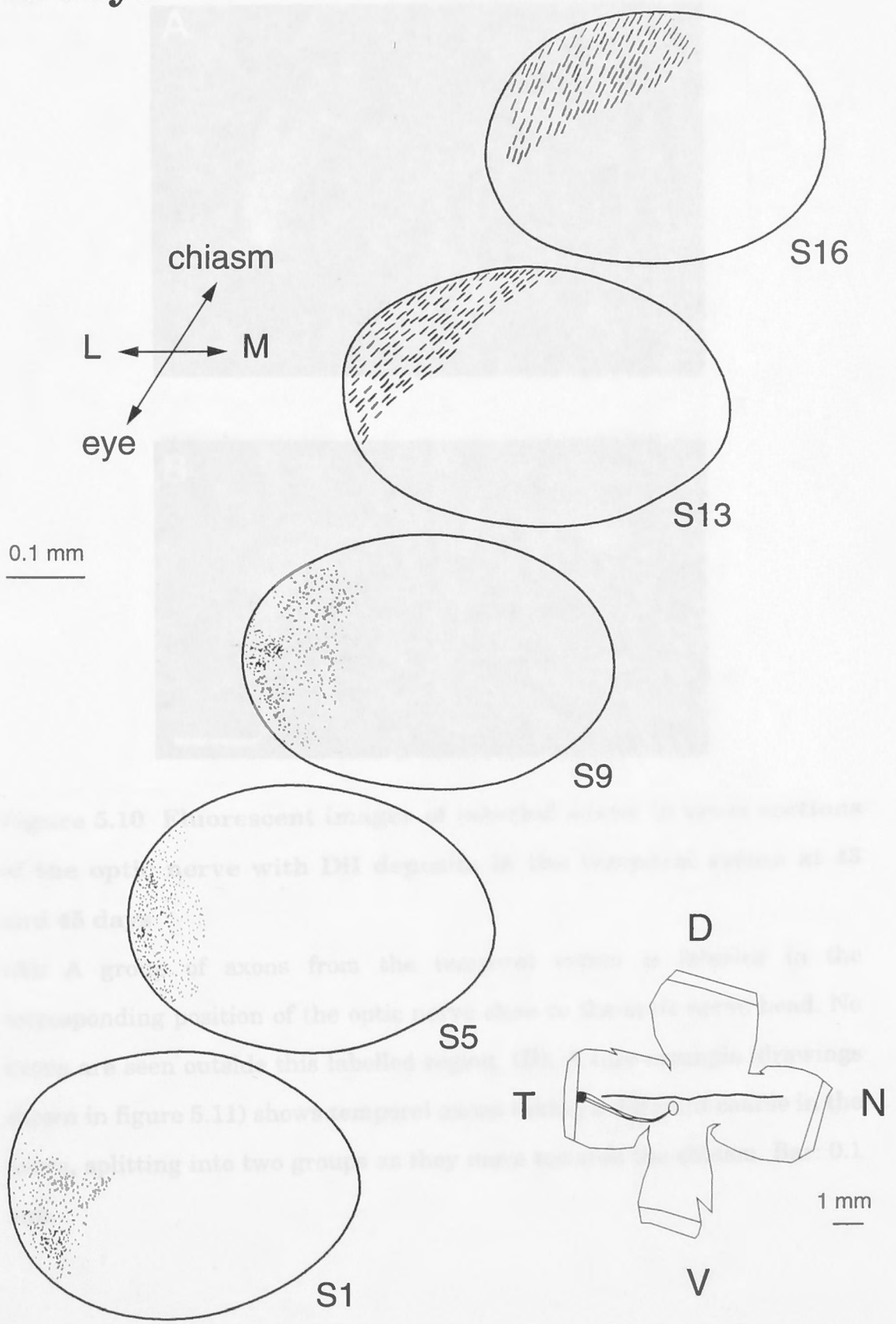


Figure 5.9 Line drawings of cross sections of the optic nerve with a DiI deposit in the temporal retina at 43 days

Conventions are the same as for figure 5.1 and 5.3. Behind the optic head, labelled temporal axons are confined to a region in the corresponding position of the optic nerve (S1). When the axons run along the nerve from the optic nerve head to the chiasm, they disperse slightly and shift dorsally (S5, S9, S13, S16). A small deposit of DiI is in the temporal retina.

43 days



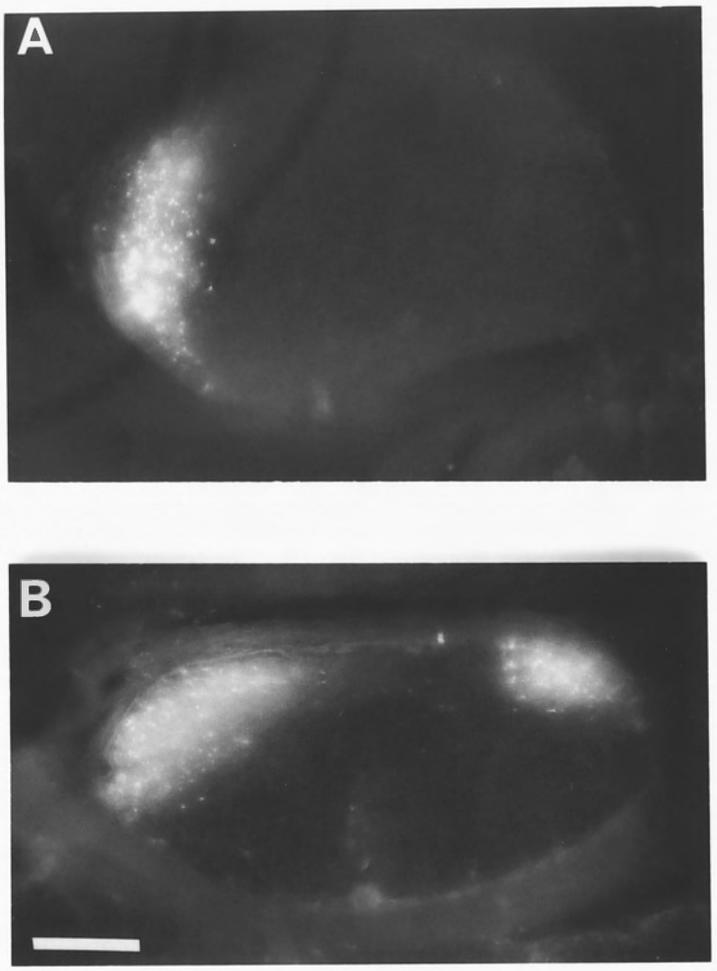


Figure 5.10 Fluorescent images of labelled axons in cross sections of the optic nerve with DiI deposits in the temporal retina at 43 and 45 days

(A): A group of axons from the temporal retina is labelled in the corresponding position of the optic nerve close to the optic nerve head. No axons are seen outside this labelled region. **(B)**: A rare example (drawings shown in figure 5.11) shows temporal axons taking a different course in the nerve, splitting into two groups as they move towards the chiasm. Bar: 0.1 mm.

Figure 5.11 Line drawings of cross sections of the optic nerve with a DiI deposit in the temporal retina at 45 days

Conventions are the same as for figure 5.1 and 5.3. This shows a rare example where temporal axons take a different course to that usually seen. Labelled axons are distributed around the periphery of the lateroventral nerve (S1) and then divide into two groups, with the main group in the lateral nerve and another small group in the medial nerve (S4). Gradually, the two groups of axons shift dorsally around the periphery of the lateral and medial nerve (S8, S11). Close to the chiasm, the axons get together to occupy a region dorsally (S15). DiI deposit is in the temporal retina.

45 days

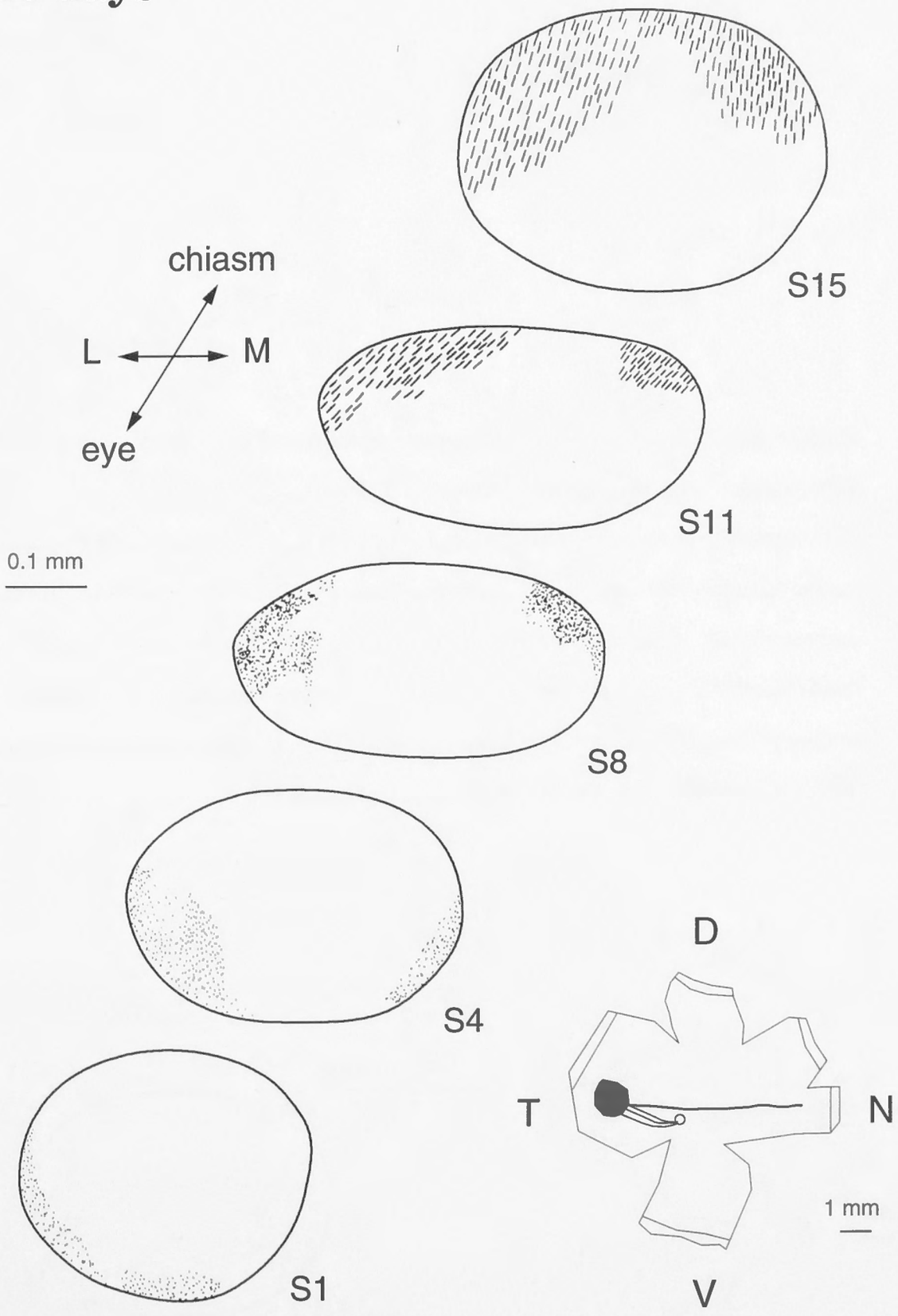
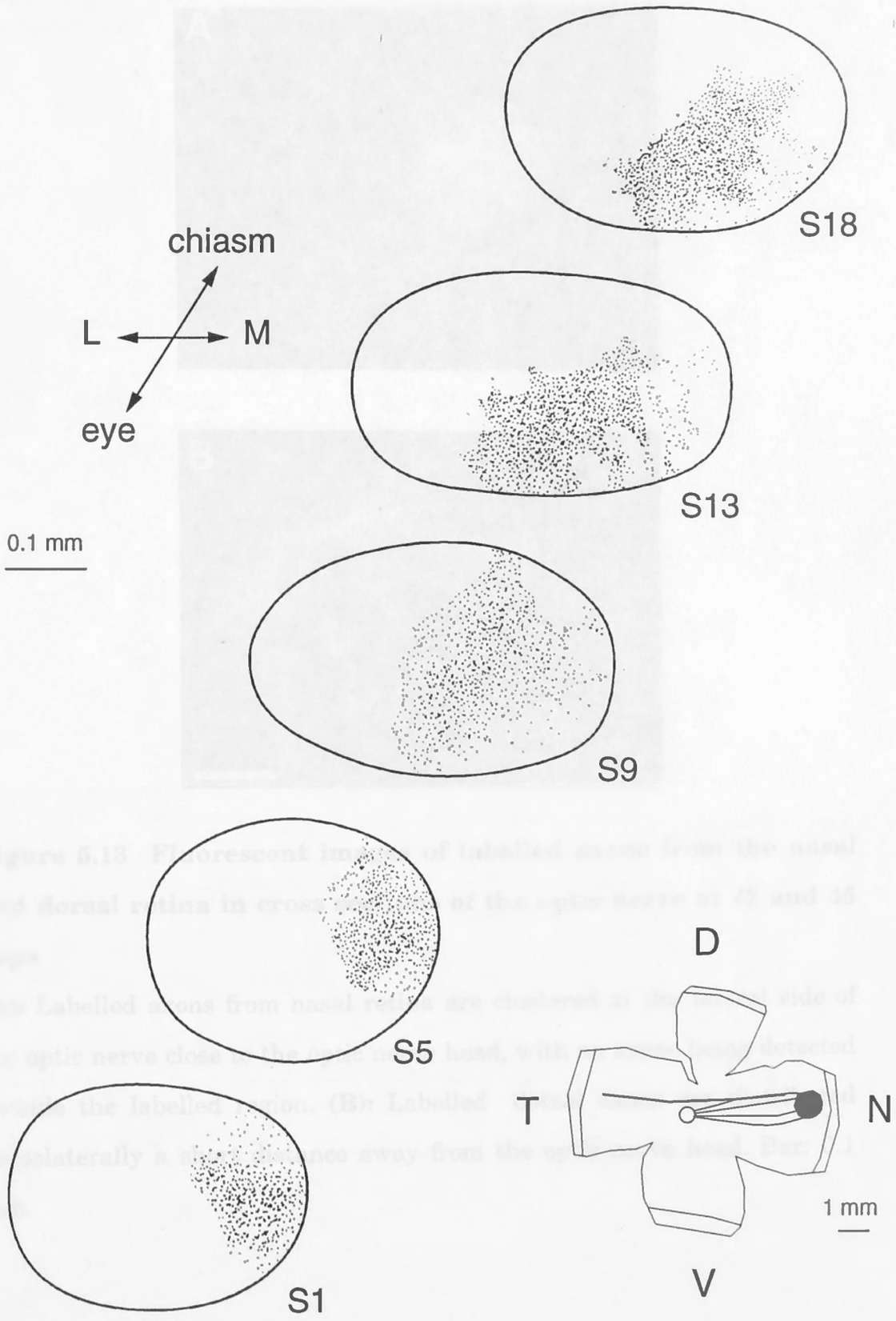


Figure 5.12 Line drawings of cross sections of the optic nerve with a DiI deposit in the nasal retina at 45 days

Conventions are the same as for figure 5.1 and 5.3. After entering the optic nerve, axons labelled by DiI in the nasal retina are present in the corresponding side of the nerve (S1). Gradually, the region occupied by nasal axons in the optic nerve shifts slightly more ventrally and axons are scattered more diffusely as distance from the optic nerve head increases (S5, S9, S13, S18). DiI deposit is seen in the nasal retina.

45 days



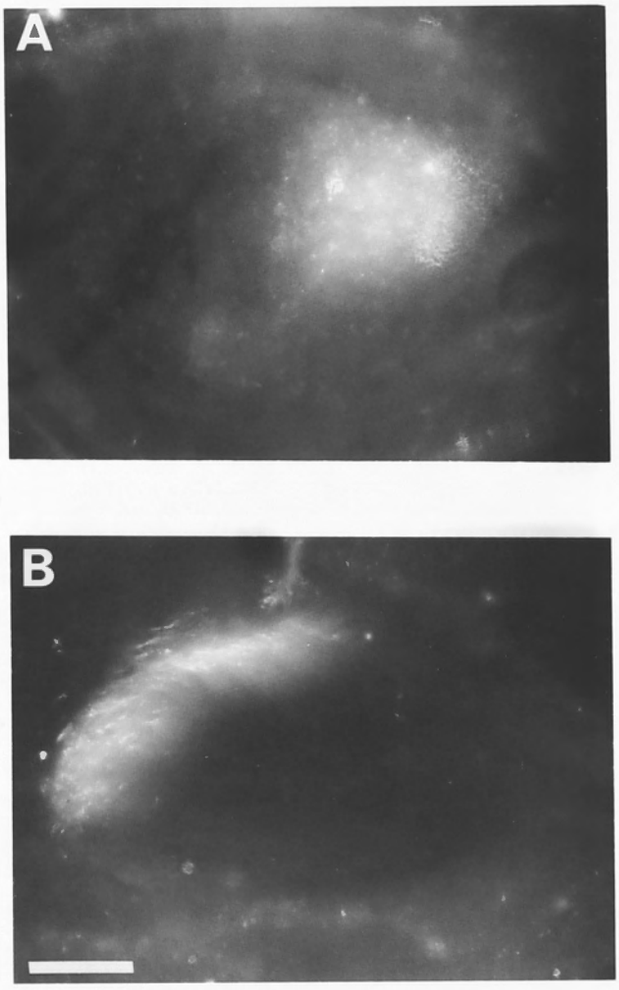


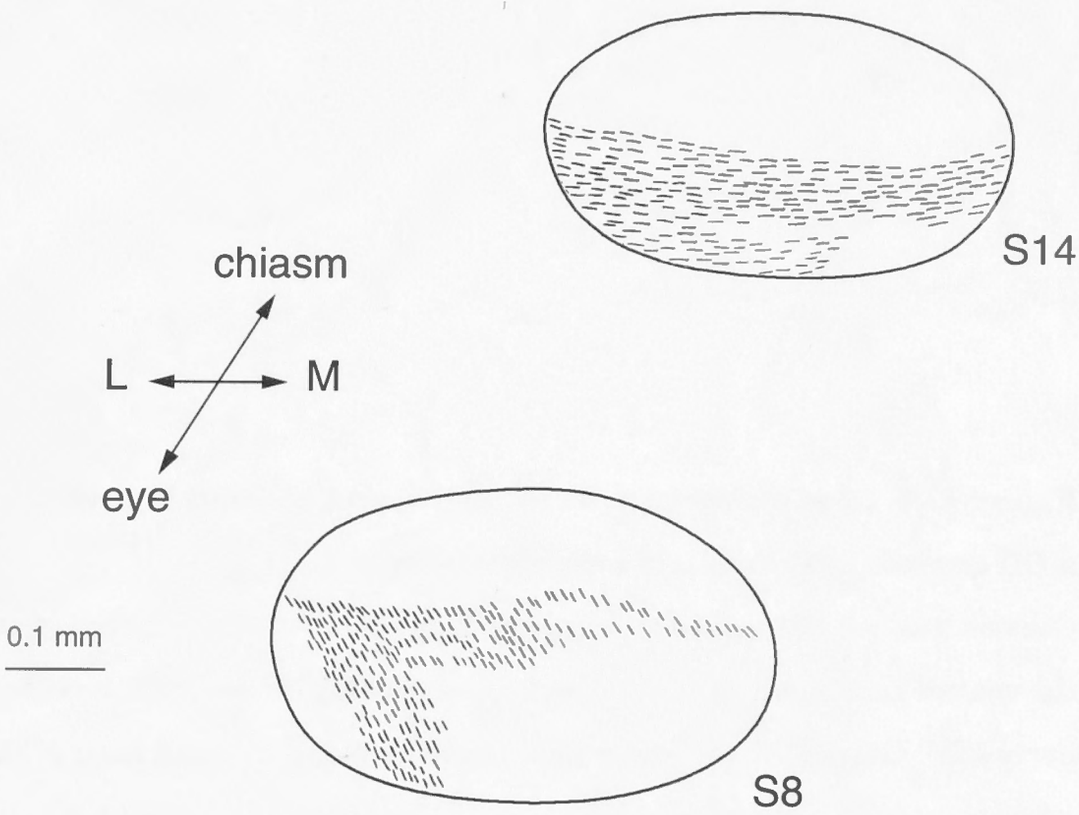
Figure 5.13 Fluorescent images of labelled axons from the nasal and dorsal retina in cross sections of the optic nerve at 42 and 45 days

(A): Labelled axons from nasal retina are clustered at the lateral side of the optic nerve close to the optic nerve head, with no axons being detected outside the labelled region. **(B):** Labelled dorsal axons are distributed dorsolaterally a short distance away from the optic nerve head. Bar: 0.1 mm.

Figure 5.14 Line drawings of cross sections of the optic nerve with a DiI deposit in the dorsal retina at 42 days

Conventions are the same as for figure 5.1 and 5.3. Axons labelled by the dye in the dorsal retina are scattered around the periphery of the dorsolateral nerve a short distance away from the optic nerve head (S2). The axons gradually shift towards the ventral side by midway along the optic nerve (S8). Prior to the chiasm, the axons are distributed diffusely in the ventral part of the optic nerve (S14). A focal DiI deposit is localized in the dorsal retina.

42 days



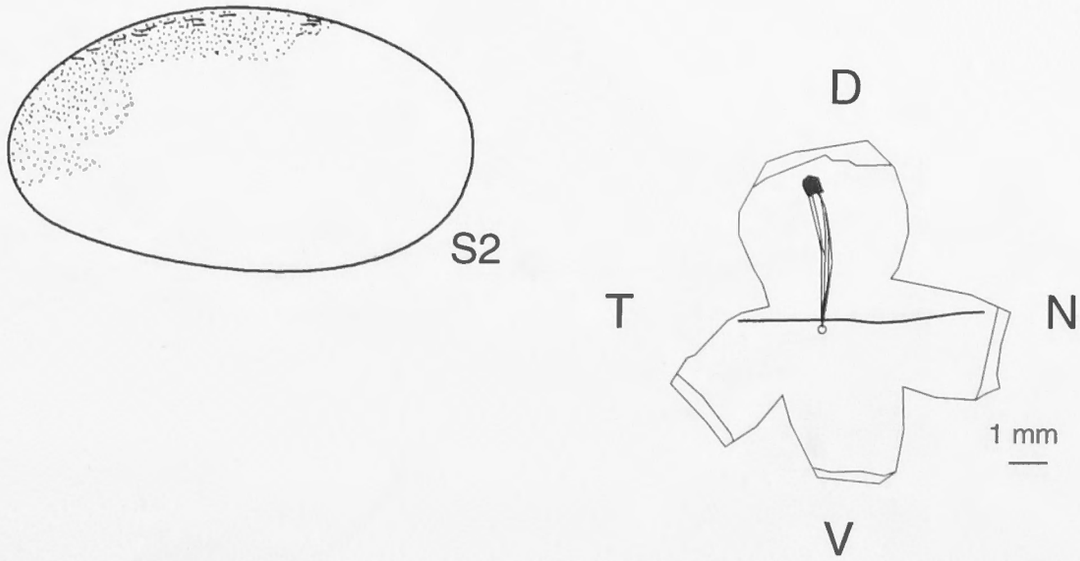
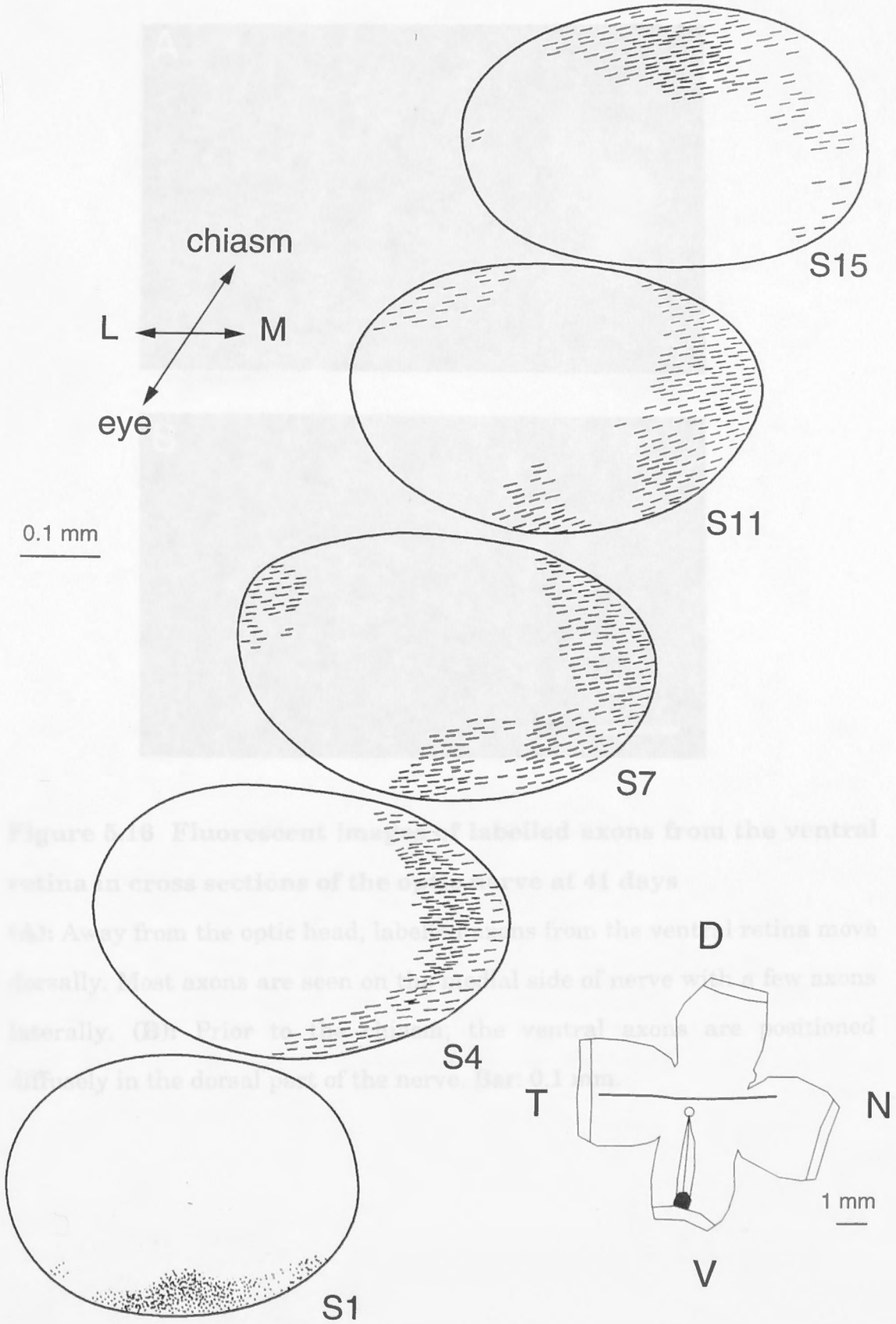


Figure 5.15 Line drawings of cross sections of the optic nerve with a DiI deposit in the ventral retina at 41 days

Conventions are the same as for figure 5.1 and 5.3. As labelled axons from the ventral retina enter the optic nerve, they occupy an area in the ventral nerve (S1). Gradually, the axons move dorsally along the periphery of the nerve, mainly in the medial side (S4, S8, S11). Axons are distributed more diffusely in a region of the dorsal part of the optic nerve just before the chiasm (S15). A ventral deposit of DiI in the retina is seen.

41 days



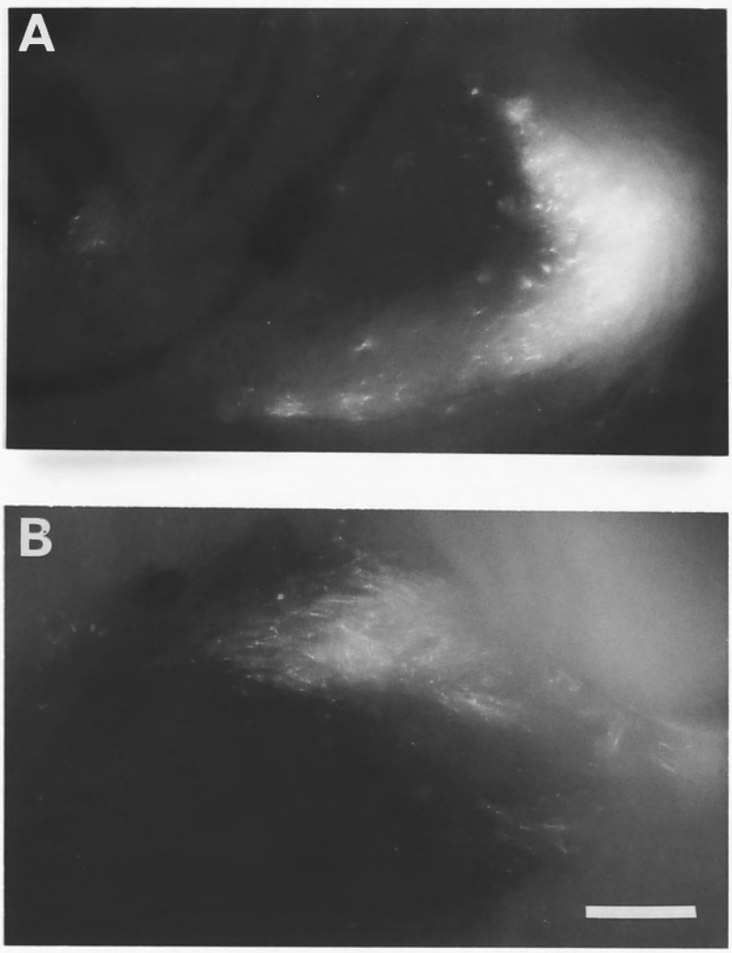


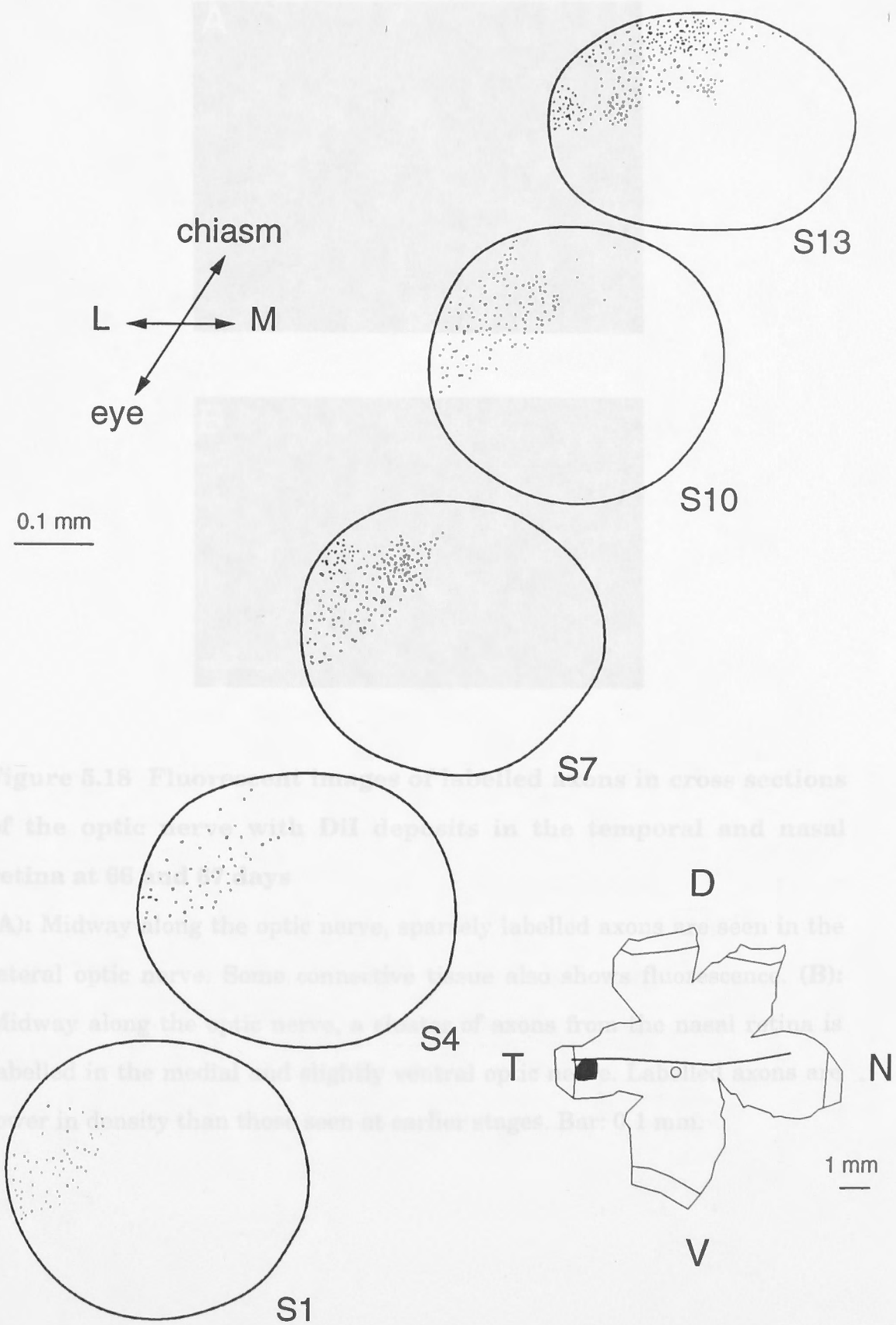
Figure 5.16 Fluorescent images of labelled axons from the ventral retina in cross sections of the optic nerve at 41 days

(A): Away from the optic head, labelled axons from the ventral retina move dorsally. Most axons are seen on the medial side of nerve with a few axons laterally. **(B):** Prior to the chiasm, the ventral axons are positioned diffusely in the dorsal part of the nerve. Bar: 0.1 mm.

Figure 5.17 Line drawings of cross sections of the optic nerve with a DiI deposit in the temporal retina at 67 days

Conventions are the same as for figure 5.1 and 5.3. Axons labelled by DiI in the temporal retina are distributed sparsely in a confined region of the lateral nerve behind the optic nerve head (S1). The temporal axons shift slightly dorsally along the optic nerve (S4, S7, S10) and are scattered more diffusely in the temporodorsal part close to the chiasm (S13). The DiI deposit is found in the temporal retina.

67 days



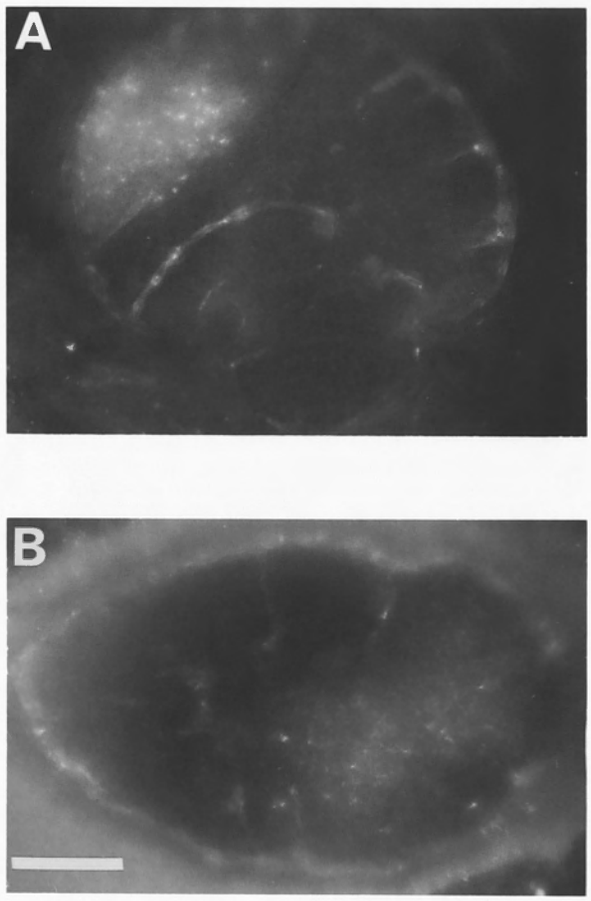


Figure 5.18 Fluorescent images of labelled axons in cross sections of the optic nerve with DiI deposits in the temporal and nasal retina at 66 and 67 days

(A): Midway along the optic nerve, sparsely labelled axons are seen in the lateral optic nerve. Some connective tissue also shows fluorescence. **(B):** Midway along the optic nerve, a cluster of axons from the nasal retina is labelled in the medial and slightly ventral optic nerve. Labelled axons are lower in density than those seen at earlier stages. Bar: 0.1 mm.

Figure 5.19 Line drawings of cross sections of the optic nerve with a DiI deposit in the nasal retina at 66 days

Conventions are the same as for figure 5.1 and 5.3. Close to the optic nerve head, labelled axons from the nasal retina are confined to the corresponding position of the nerve (S2). These axons gradually migrate slightly ventrally (S5, S8, S11) and are positioned more diffusely in the nasoventral part of the nerve prior to the chiasm (S13). DiI deposit is in the nasal retina.

66 days

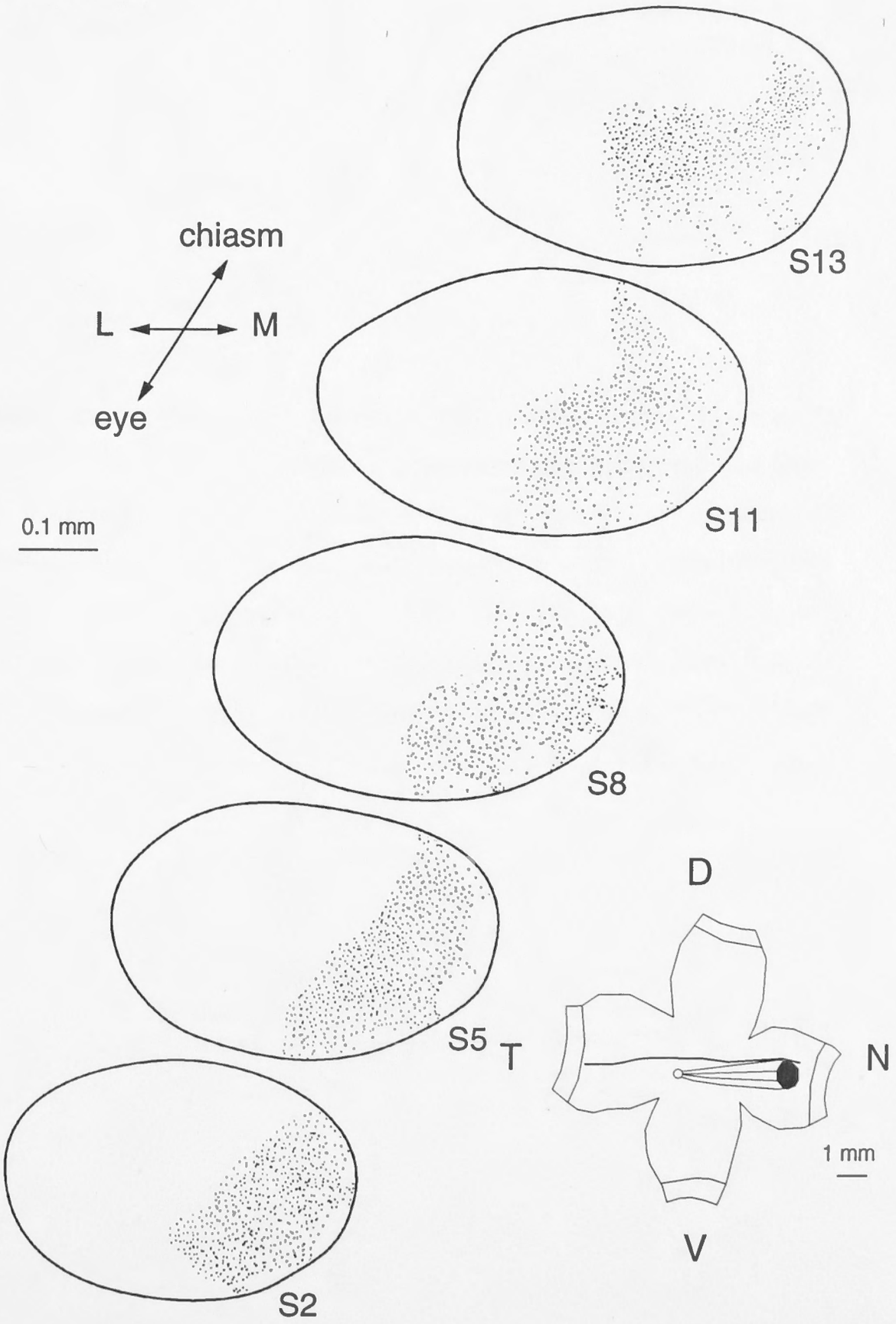
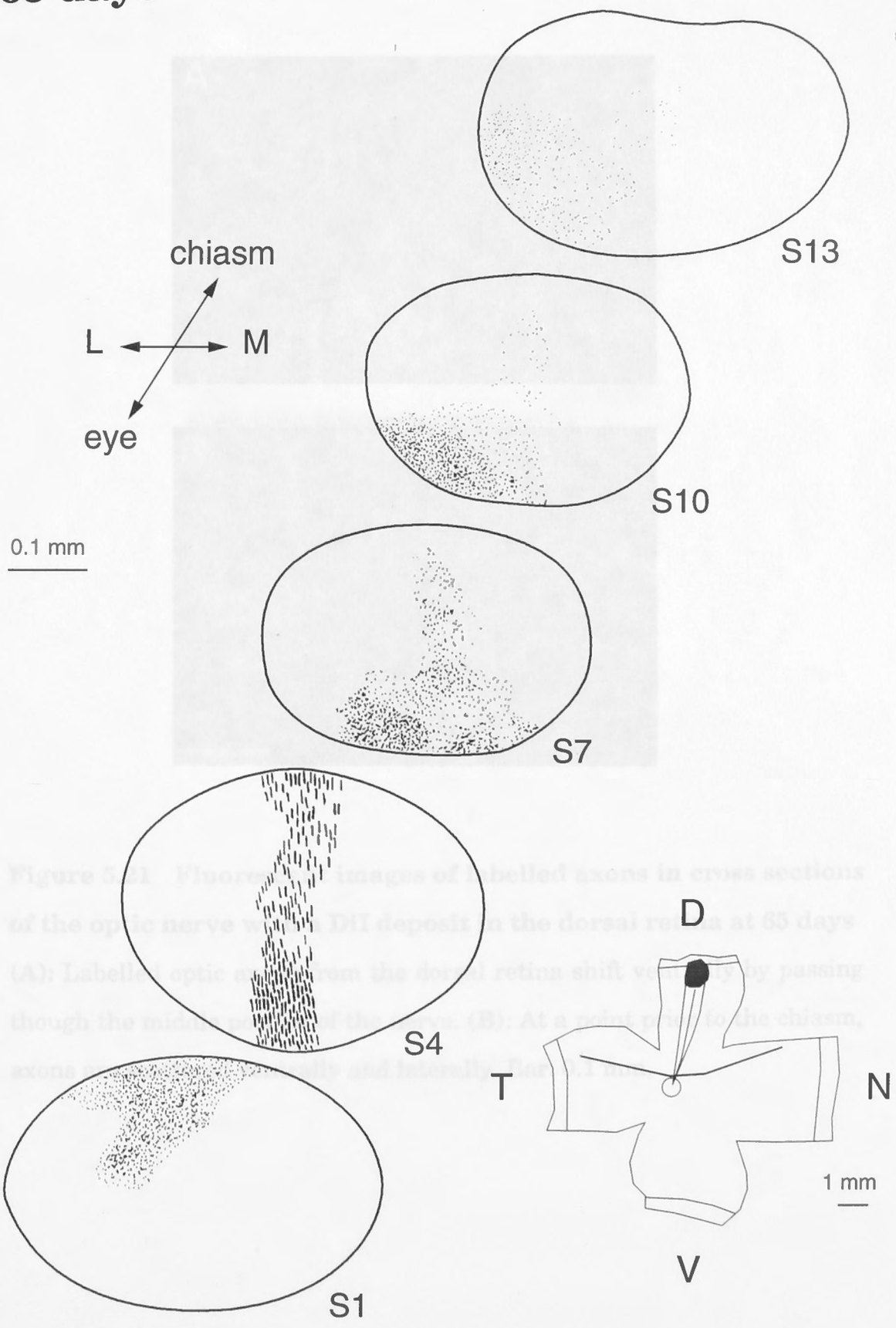


Figure 5.20 Line drawings of cross sections of the optic nerve from a DiI deposit in the dorsal retina at 65 days

Conventions are the same as for figure 5.1 and 5.3. Labelled axons from the dorsal retina enter the corresponding region of the optic nerve close to the optic nerve head (S1). Then the axons gradually migrate ventrally through the middle portion (S4, S7, S10) to occupy more diffusely a ventral, slightly lateral position close to the chiasm (S13). A defined deposit of DiI is seen in the dorsal retina.

65 days



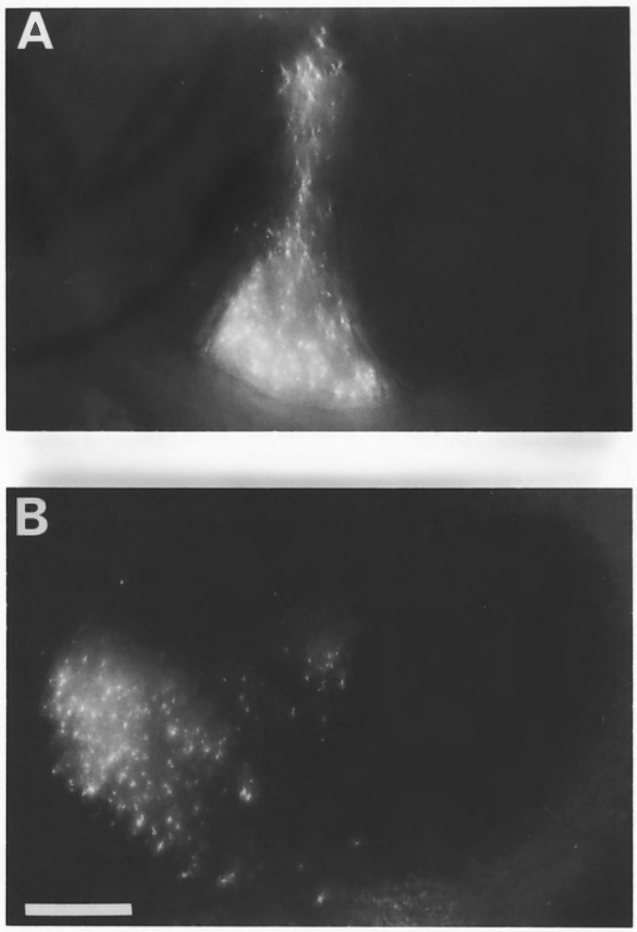
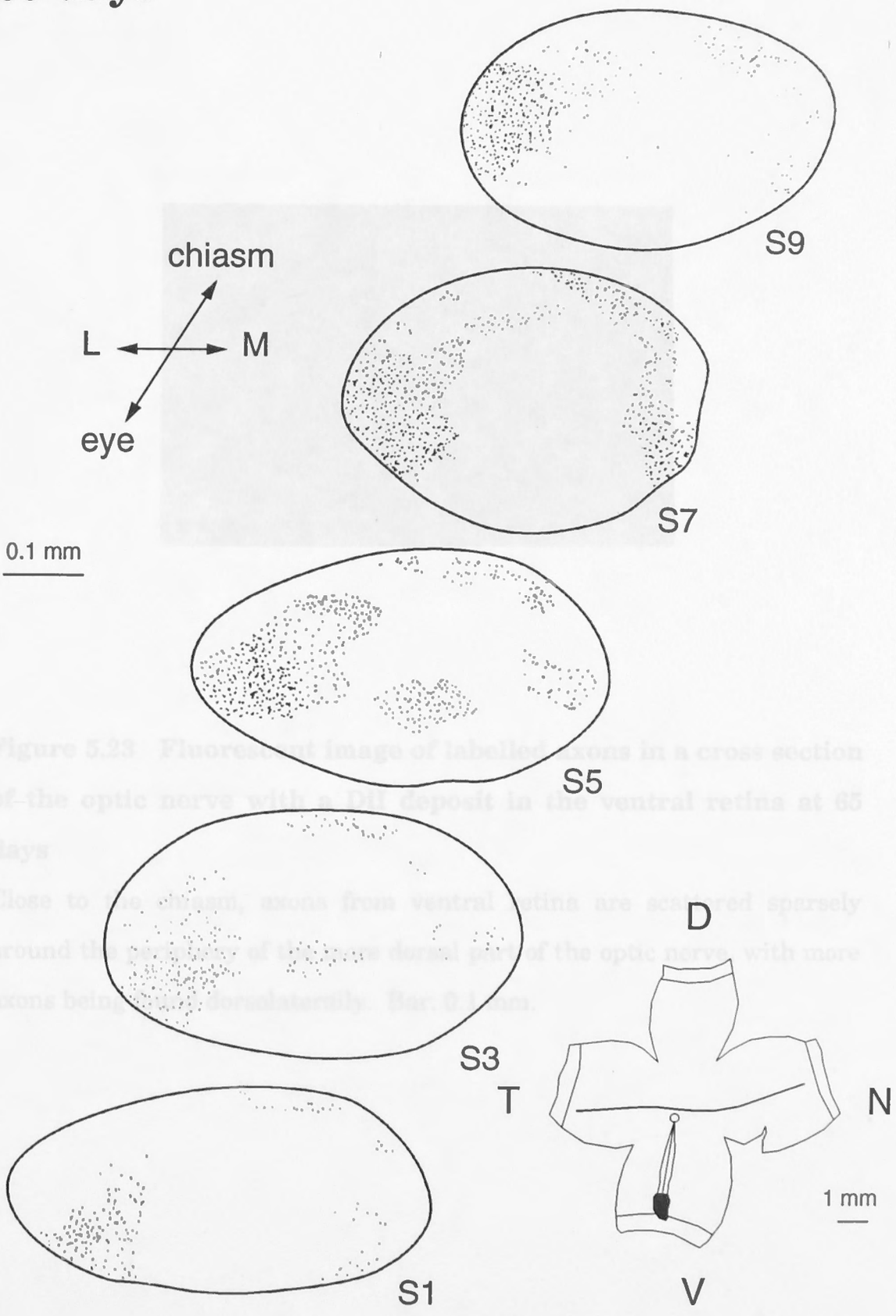


Figure 5.21 Fluorescent images of labelled axons in cross sections of the optic nerve with a DiI deposit in the dorsal retina at 65 days **(A):** Labelled optic axons from the dorsal retina shift ventrally by passing though the middle portion of the nerve. **(B)**: At a point prior to the chiasm, axons are scattered ventrally and laterally. Bar: 0.1 mm.

Figure 5.22 Line drawings of cross sections of the optic nerve with a DiI deposit in the ventral retina at 65 days

Conventions are the same as for figure 5.1 and 5.3. At a point shortly away from the optic head, labelled axons from the ventral retina start to move towards the opposite side of the nerve by splitting primarily into two groups (S1). Although a small number of axons gradually shifts dorsally through the central regions of the nerve, most axons are distributed diffusely in a crescent shaped region in the dorsal nerve as the optic nerve approaches the chiasm (S3, S5, S7, S9). The DiI deposit is in the ventral retina.

65 days



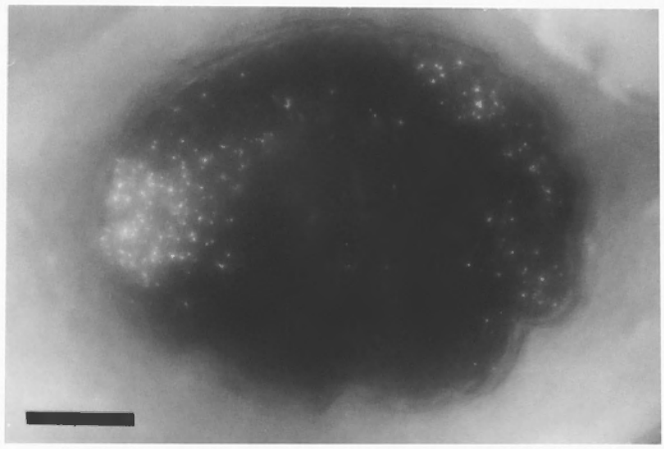


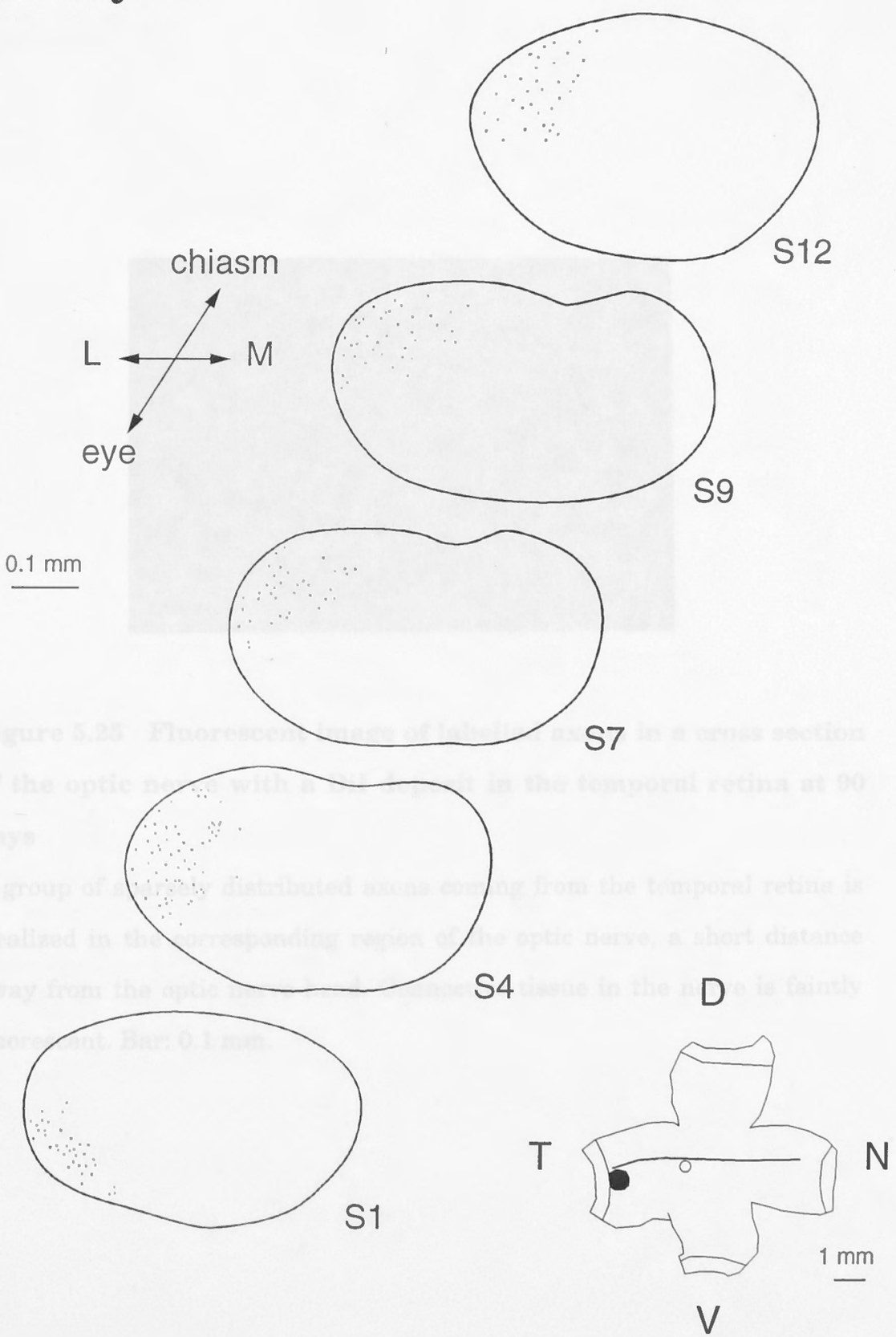
Figure 5.23 Fluorescent image of labelled axons in a cross section of the optic nerve with a DiI deposit in the ventral retina at 65 days

Close to the chiasm, axons from ventral retina are scattered sparsely around the periphery of the more dorsal part of the optic nerve, with more axons being found dorsolaterally. Bar: 0.1 mm.

Figure 5.24 Line drawings of cross sections of the optic nerve with a DiI deposit in the temporal retina at 90 days

Conventions are the same as for figure 5.1 and 5.3. Very sparse axons labelled by DiI in the temporal retina are confined to the corresponding position of the nerve behind the optic head (S1). Gradually, the temporal axons slightly shift more dorsally, with increasing distance from the optic head (S4, S7, S9, S12). A focal DiI deposit is localized in the temporal retina.

90 days



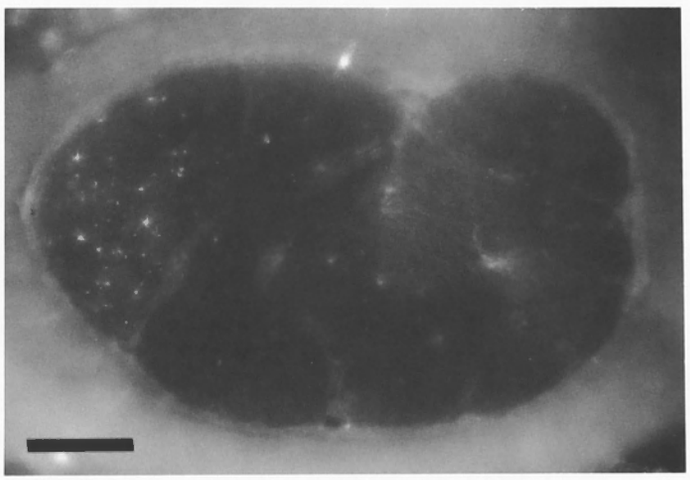


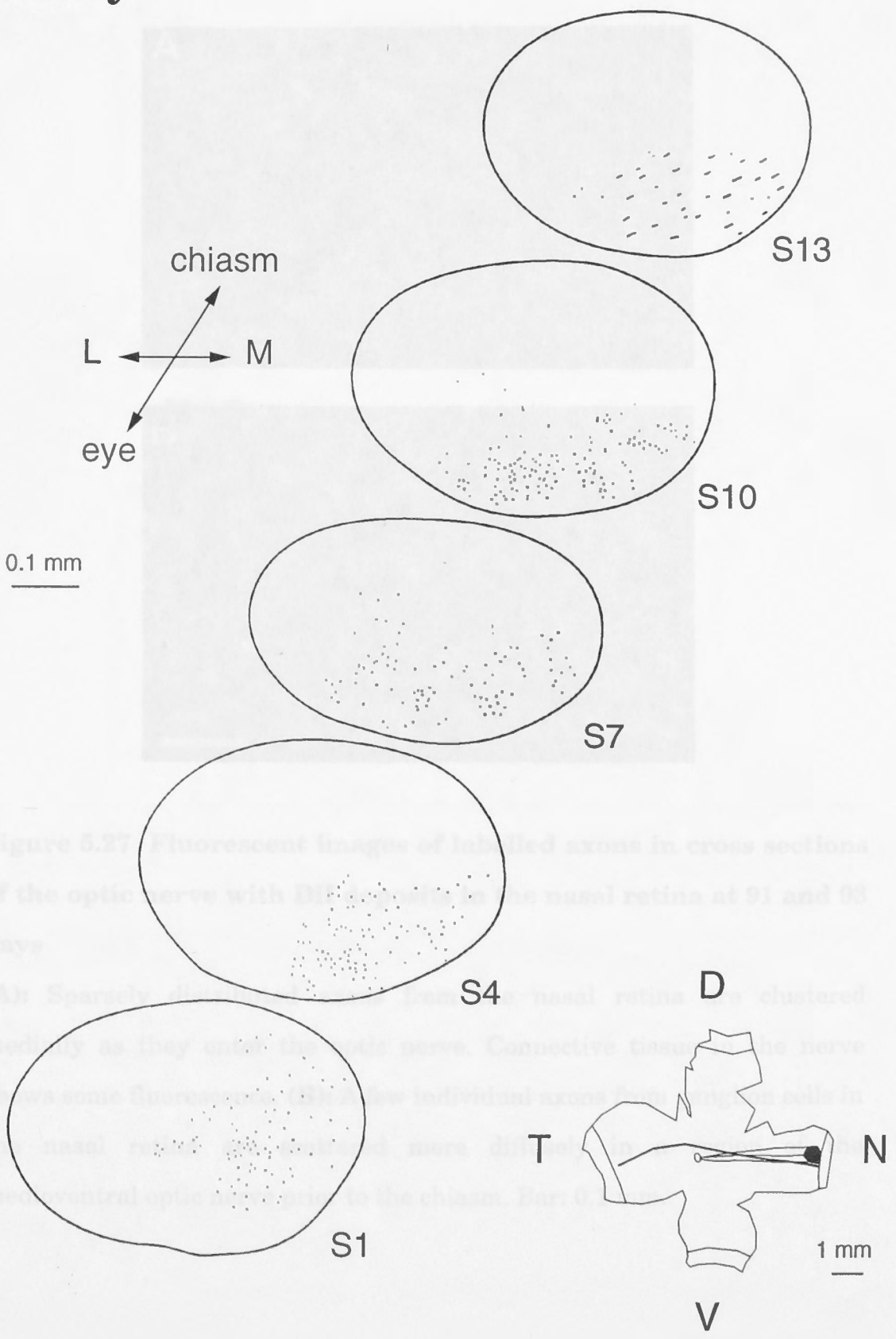
Figure 5.25 Fluorescent image of labelled axons in a cross section of the optic nerve with a DiI deposit in the temporal retina at 90 days

A group of sparsely distributed axons coming from the temporal retina is localized in the corresponding region of the optic nerve, a short distance away from the optic nerve head. Connective tissue in the nerve is faintly fluorescent. Bar: 0.1 mm.

Figure 5.26 Line drawings of cross sections of the optic nerve with a DiI deposit in the nasal retina at 91 days

Conventions are the same as for figure 5.1 and 5.3. As labelled axons from the nasal retina enter the optic nerve, they are distributed sparsely in the corresponding side of the nerve (S1). The nasal axons shift gradually medioventrally along the optic nerve (S4, S7, S10). Finally, the axons are positioned medioventrally before the chiasm. (S13). A DiI deposit is found in the periphery of the nasal retina.

91 days



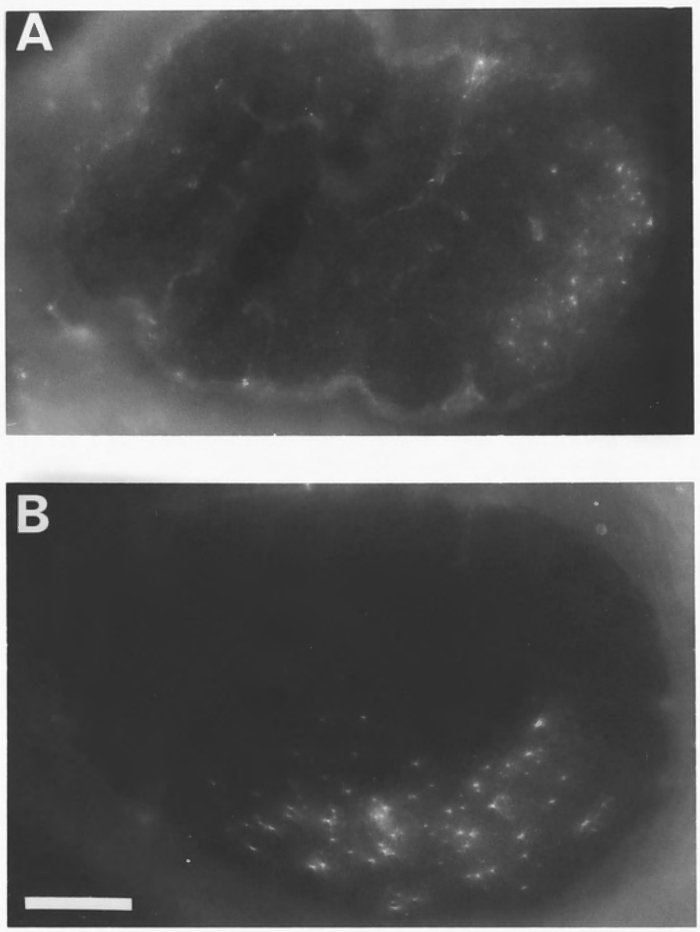


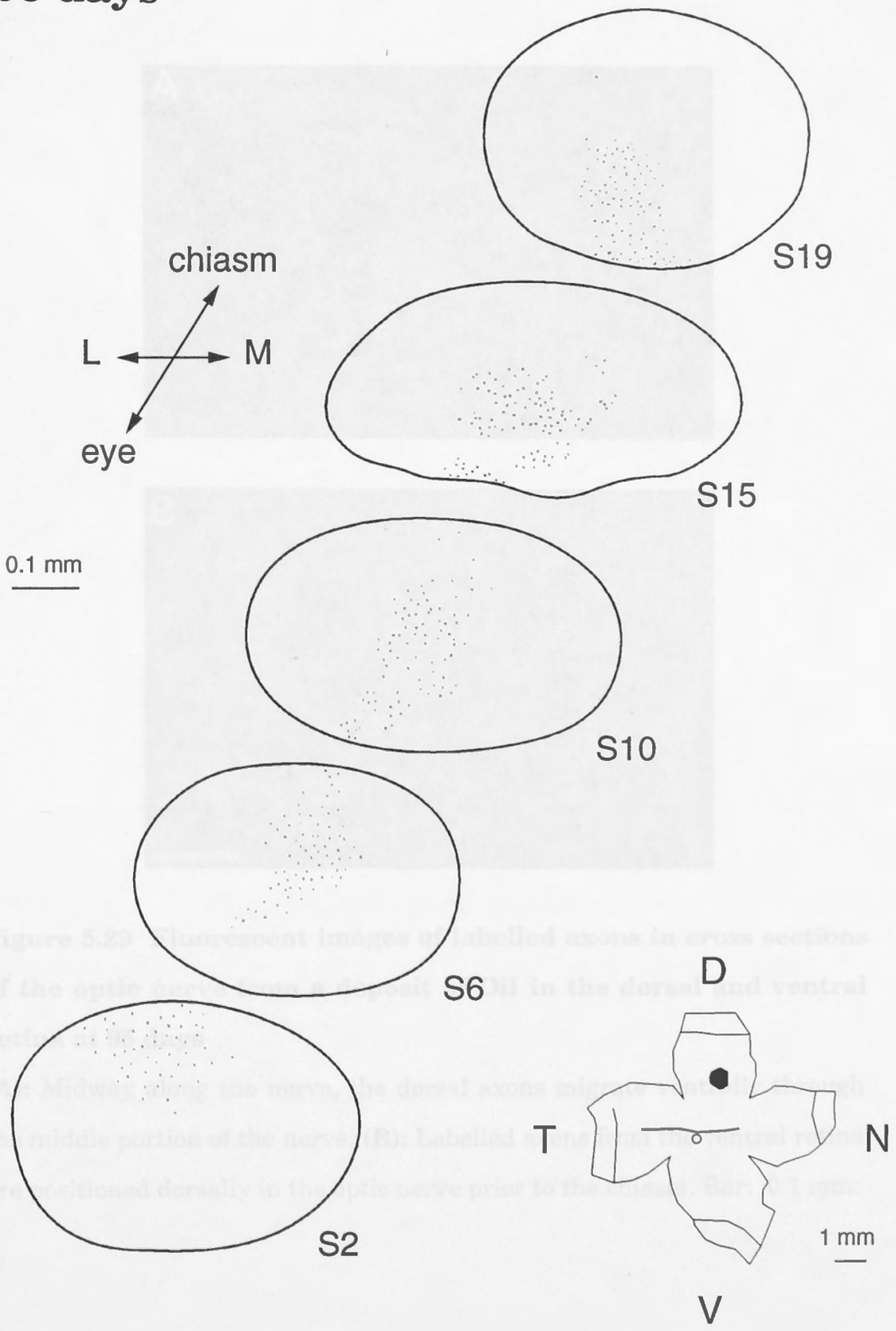
Figure 5.27 Fluorescent images of labelled axons in cross sections of the optic nerve with DiI deposits in the nasal retina at 91 and 93 days

(A): Sparsely distributed axons from the nasal retina are clustered medially as they enter the optic nerve. Connective tissue in the nerve shows some fluorescence. **(B):** A few individual axons from ganglion cells in the nasal retina are scattered more diffusely in a region of the medioventral optic nerve prior to the chiasm. Bar: 0.1 mm.

Figure 5.28 Line drawings of cross sections of the optic nerve with a DiI deposit in the dorsal retina at 95 days

Conventions are the same as for figure 5.1 and 5.3. After entering the optic nerve, sparsely labelled axons from the dorsal retina are clustered dorsally (S2). Subsequently, the dorsal axons migrate gradually towards the opposite side, through the middle portion of the nerve (S6, S10, S15). Finally, the dorsal axons are scattered in the ventral optic nerve (S19). A deposit of DiI is in more central dorsal retina.

95 days



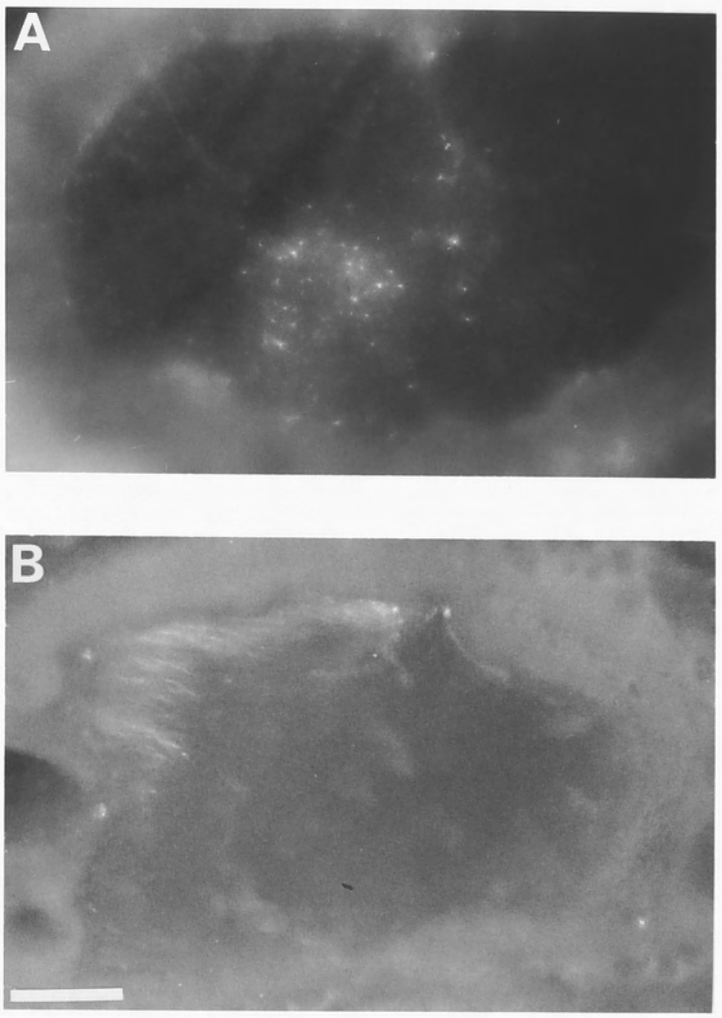


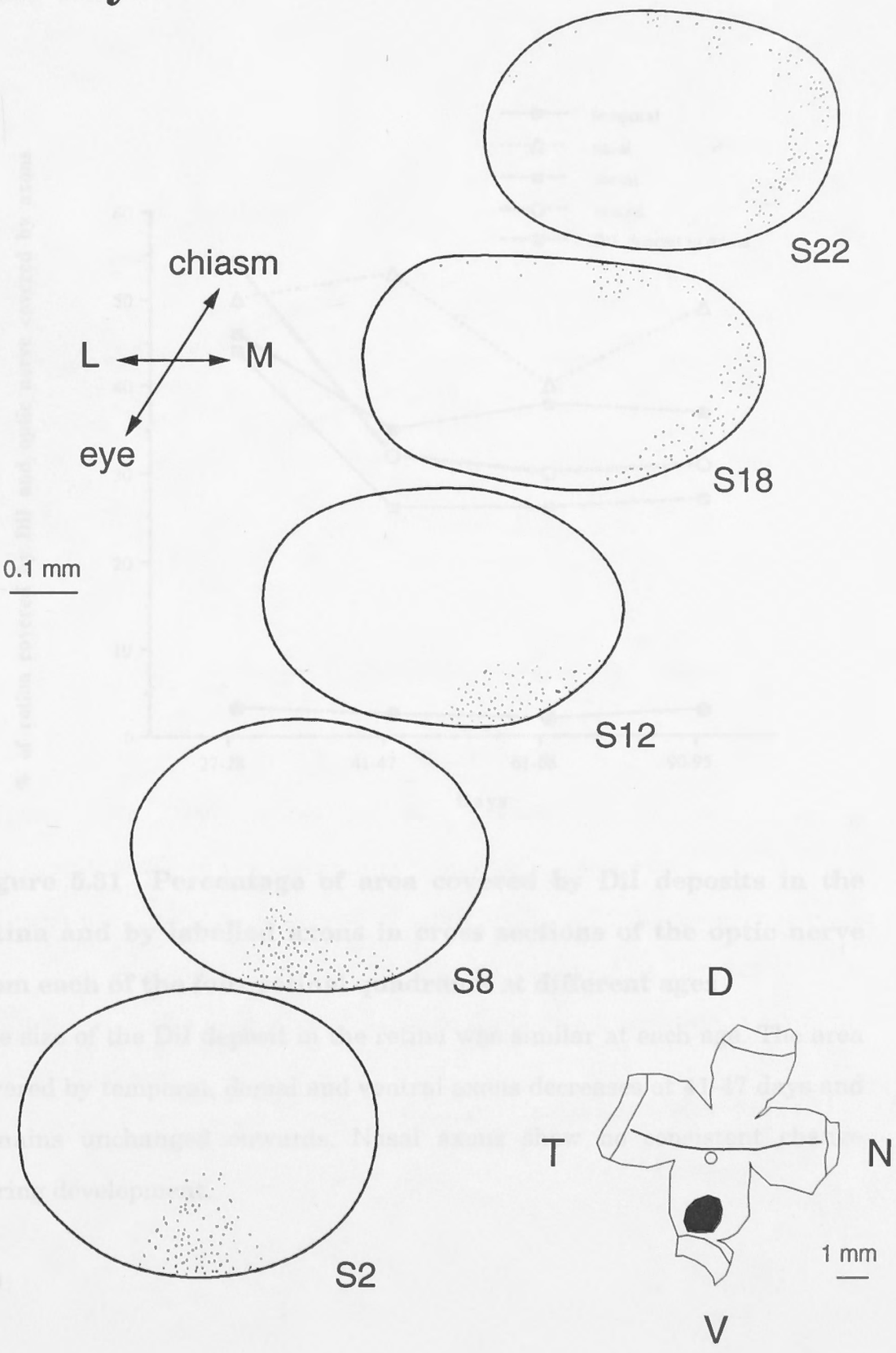
Figure 5.29 Fluorescent images of labelled axons in cross sections of the optic nerve from a deposit of DiI in the dorsal and ventral retina at 95 days

(A): Midway along the nerve, the dorsal axons migrate ventrally through the middle portion of the nerve. **(B):** Labelled axons from the ventral retina are positioned dorsally in the optic nerve prior to the chiasm. Bar: 0.1 mm.

Figure 5.30 Line drawings of cross sections of the optic nerve with a DiI deposit in the ventral retina at 93 days

Conventions are the same as for figure 5.1 and 5.3. Labelled axons from the ventral retina are distributed sparsely in a confined region of the ventral nerve behind the optic nerve head (S2). The axons move gradually to the opposite side along the medial border of the optic nerve (S8, S12, S18). Prior to the chiasm, the axons are spread diffusely in a crescent shaped region mediodorsally (S22). A large deposit of DiI is in the ventral retina.

93 days



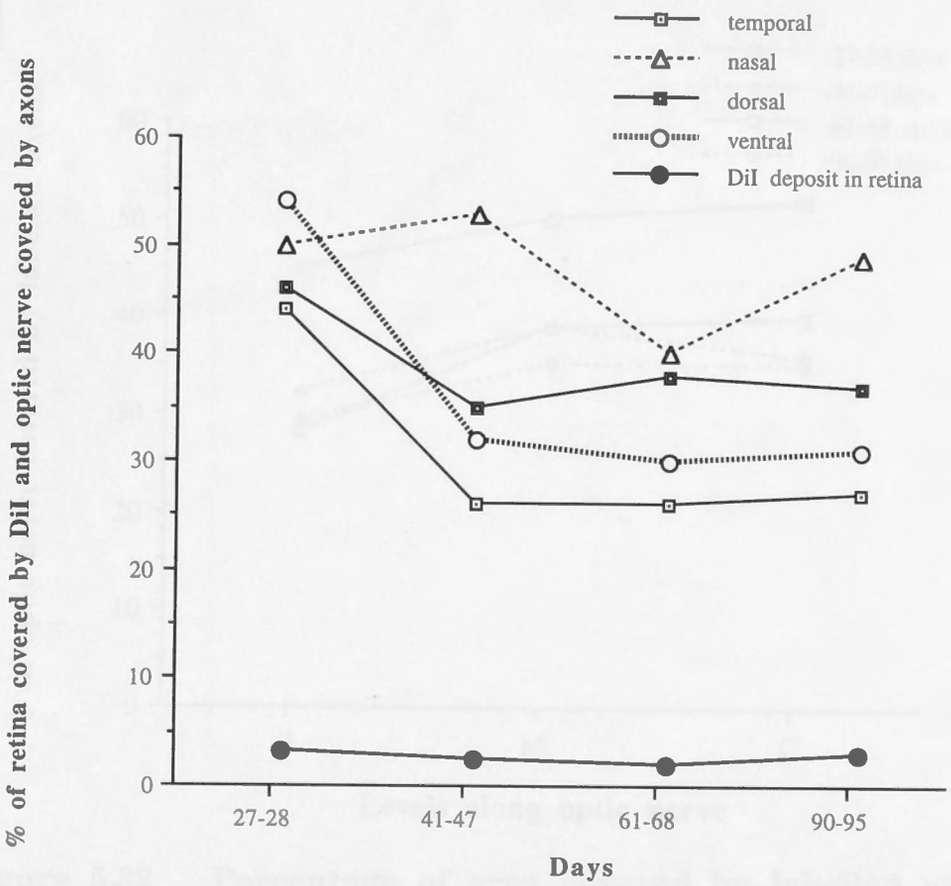


Figure 5.31 Percentage of area covered by DiI deposits in the retina and by labelled axons in cross sections of the optic nerve from each of the four retinal quadrants at different ages

The size of the DiI deposit in the retina was similar at each age. The area covered by temporal, dorsal and ventral axons decreases at 41-47 days and remains unchanged onwards. Nasal axons show no consistent change during development.

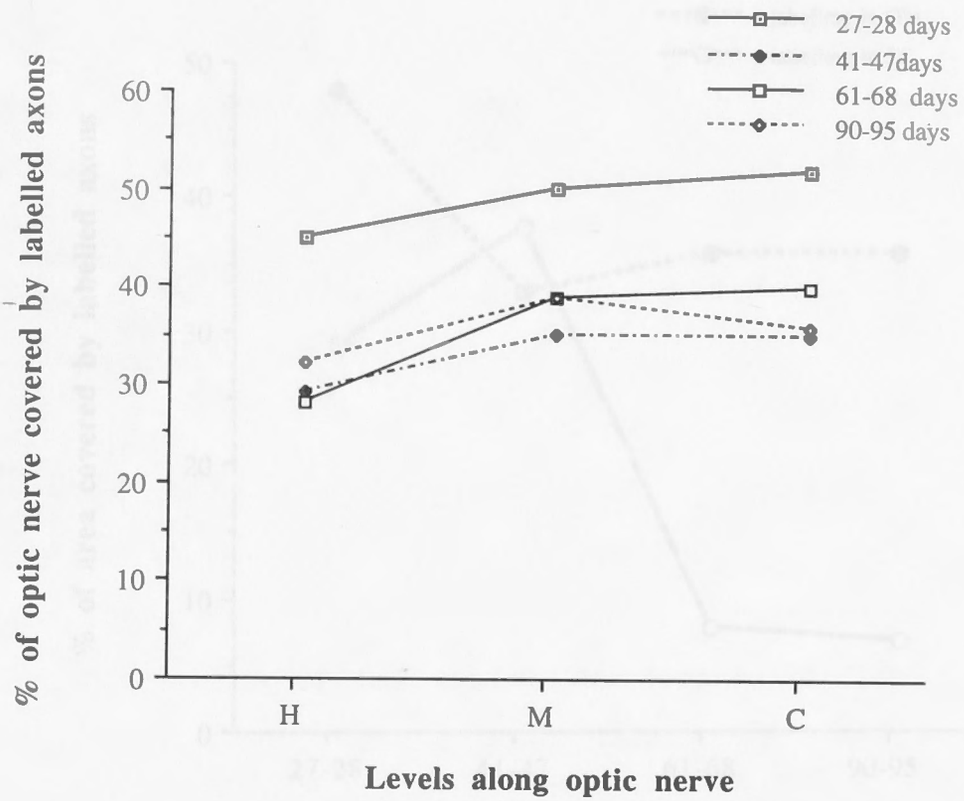


Figure 5.32 Percentage of area covered by labelled axons at different levels of the optic nerve for each age group

At each age, the region covered by labelled axons in the nerve, pooled for all retinal quadrants, increases slightly with increasing distance from the optic nerve head to the chiasm. H: optic nerve head; M: midway along the nerve; C: chiasm.

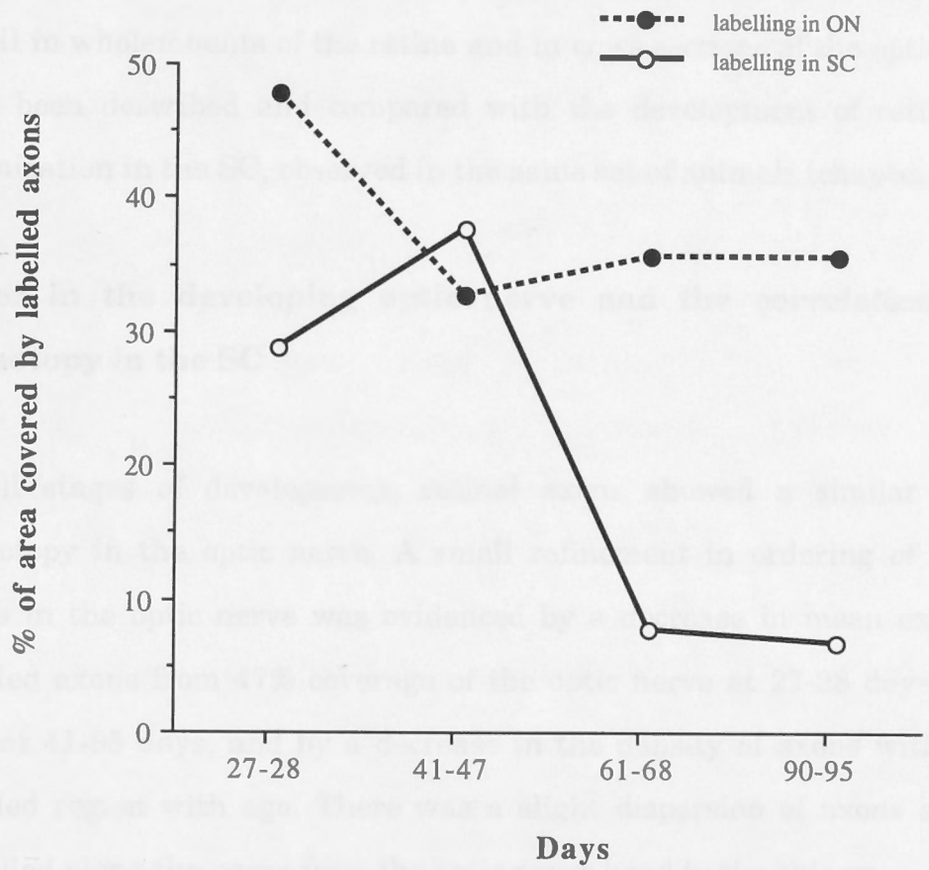


Figure 5.33 Graph of pooled data for all retinal quadrants at each age showing the relationship in retinotopic organization between the optic nerve and the SC

The small decrease in the area occupied by retinal axons in the optic nerve at 41-47 days does not correlate with the dramatic refinement in the retinal projection in the SC at 61-68 days.

DISCUSSION

To examine the retinotopic order in the developing optic nerve of the wallaby *(Macropus eugenii),* the course and organization of axons labelled by DiI in wholemounts of the retina and in cross sections of the optic nerve have been described and compared with the development of retinotopic organization in the SC, observed in the same set of animals (chapter 4).

Order in the developing optic nerve and the correlation with retinotopy in the SC

At all stages of development, retinal axons showed a similar coarse retinotopy in the optic nerve. A small refinement in ordering of retinal axons in the optic nerve was evidenced by a decrease in mean extent of labelled axons from 47% coverage of the optic nerve at 27-28 days to 32-35% at 41-95 days, and by a decrease in the density of axons within the labelled region with age. There was a slight dispersion of axons as they travelled along the nerve from the optic nerve head to the chiasm.

At 27-28 days, temporal axons remained on the corresponding side of the optic nerve laterally and the nasal axons on the medial side. The dorsal axons gradually migrated from the corresponding dorsal region towards the opposite side of the optic nerve by passing through the middle portion of the nerve. Two different patterns were found from the ventral axons as they shifted dorsally in the optic nerve. In some cases, the ventral axons shifted towards the dorsal side of the nerve in one group along one side of the optic nerve. In a similar number of other cases, when the peripherally located ventral axons ascended to the dorsal region, they split into two groups. Temporal axons were also found, in one case, to split into two

groups. Whether or not axons split into two groups is presumably due to their course from peripheral retina to the optic disc in relation to the remnant of the temporoventrally located optic fissure. Before the axons from dorsal and ventral retina reached the chiasm, they were positioned on opposite sides of the nerve. At the same developmental stage, retinal axons in the SC were also distributed in a rough retinotopic ordering, with the temporal axons projecting rostrally, the nasal axons caudally, dorsal axons laterally and ventral axons medially (chapter 4). By 41-47 days, when a slightly higher degree of retinotopic order was seen in the optic nerve, the retinocollicular projection advanced dramatically with terminal aborizations being formed by temporal axons in the correct topographic region (chapter 4). From 61-95 days, there was an obvious decease in the density of labelled axons in the nerve. This reduction in density is consistent with ganglion cell death seen in the retina during these stages (Spira and Marotte, '89). However, the degree of topographic ordering of retinal axons in the optic nerve remained unchanged. Retinal axons still covered a similar area of the nerve to that seen at earlier stage. At these stages, the retinocollicular projection underwent a significant refinement. Many of initially more widely distributed axons disappeared and mature discrete terminal zones were present with both the absolute size of terminal zones and their percentage coverage of the SC declining (chapter 4).

Retinal axons in the optic nerve displayed a limited degree of retinotopic order, with only a small change at different developmental stages. The degree of the retinotopic order only slightly increased with age. This coarse retinotopic order in the nerve up to 41 days is likely to assist, partly at least, in distributing axons in coarse retinotopic order in the SC during the first stage of the retinocollicular projection when axons from the dorsal and

ventral retina innervated the lateral and medial SC, respectively. In the optic nerve, axons from the dorsal retina moved to a ventral position while axons from the ventral retina moved to a dorsal position. This would lead to dorsal axons being lateral and ventral axons being medial in the optic tract. This is in fact what is seen as these axons approach the SC. That is, they are positioned appropriately in the tract to approach the region of the SC they innervate. However, no evidence demonstrated a similar event in the axonal innervation from the temporal and nasal retina. Temporal and nasal axons, although they were also distributed in an orderly fashion in the optic nerve, entered the SC across the whole width of the optic tract. This position in the optic tract can not explain the different distribution of the temporal and nasal axons in the SC. Further cues, as discussed in the previous chapter, are necessary.

Unlike the first stage in development, it seems that retinotopy in the optic nerve is not related to the development of the retinocollicular projection in the second stage when discrete terminals are made in their retinotopically correct positions in the SC. The position of axons in the optic nerve was not related to their ability to make synapses in the correct region in the SC because there was no obvious increase in the degree of retinotopy in the nerve, although axon density decreased, at the same time as the formation of terminals in the SC and the loss of more widely distributed axons. An increase in retinotopy in the optic nerve at this time would imply that the widely distributed axons in the SC had been unable to make terminals in the correct place in the SC because of their position in the nerve. This was not the case. Thus, the formation of the visual map in the SC of the wallaby cannot be explained completely by the mechanism of pre-ordering. The emergence of topographically ordered terminals in the SC is not dependent on the topography of retinal axons in the optic nerve. However,

the present findings could explain partly at least the extension of retinal axons in rough retinotopic ordering in the SC at the first stage up to 41 days, in which dorsal and ventral axons were found to enter the SC separately at the lateral and medial border, respectively.

Retinotopy in other mammalian optic nerves in relation to formation of the visual map

A similar retinal organization to that seen in the wallaby has also been obtained with similar anatomical methods in the developing optic nerve of the marsupial mammal, quokka wallaby (Chelvanavagam and Beazley, '94). Axons arising from the temporal and nasal retina maintain corresponding regions throughout the optic nerve by dominating their respective hemi-nerves. An inversion of the dorsal-ventral axis is also seen. Dorsal axons occupy a ventral position by shifting gradually from the dorsal side, and ventral axons move to a dorsal region by splitting into two groups peripherally. A similar degree of retinotopic organization to that seen in the wallaby was also found in adult rat optic nerve (Bunt and Lund, '82). The degeneration studies and retinal backfills with HRP showed that immediately behind the eye, there is an orderly retinal projection in the optic nerve and the projection becomes largely less precise as the chiasm is approached. A more detailed study (Chelvanavagam and Beazley, '92) currently showed axons from each quadrant of retina distributing in the corresponding regions as they enter the optic nerve. As in the wallaby, the temporal and nasal axons remain in the corresponding positions throughout the nerve. Dorsal axons gradually shift ventrally through the optic nerve and occupy the ventral part from the mid-point along the nerve. Ventral axons were also found to split into two groups as they ascend from a ventral to a dorsal position along the length of the optic

nerve. A dispersion of axons throughout the optic nerve was also demonstrated. The tendency for dorsal retinal axons to move ventrally in the optic nerve and ventral axons to move dorsally was also shown in normal fetal rats and mice in an earlier study (Silver and Sapiro, '81; Silver, '84). In a study on developing mouse, using retrograde tracing techniques (Colello and Guillery, '90), organization of ganglion cell axons from temporal and dorsal retina in the optic nerve was found to be similar to that seen in the wallaby. Labelled axons, mainly from the temporal retina, were distributed preferentially to the temporal half in the optic nerve, with some scattering along the length of the optic nerve. Inversion of the dorsal-ventral retinal axis was demonstrated by the dorsal axons in the optic nerve. In the study on albino rat (Simon and O'Leary, '91), however, only a much less ordered arrangement of retinal axons in the optic nerve was found. Before and after the topographic retinocollicular projection becomes well organized, a poorly organized retinotopic arrangement was seen as axons entered the optic nerve. The labelled retinal axons tended to be distributed diffusely in a specific half of the entire nerve depending upon their retinal region. Temporal and nasal axons are biased slightly towards the lateral and medial half, with dorsal and ventral toward the ventral half and dorsal half, respectively.

In other animals such as monkey and cat, although retinal axons were ordered roughly behind the optic head, a relatively great deal of scattering in the organization is observed along the length of the optic nerve, compared with that seen in the wallaby. The arrangement of retinogeniculate axons in the optic nerve in adult monkey (Naito, '89) showed that as axons from the various retinal areas enter the optic nerve, they are arranged in a wedge according to the axons' trajectory on the retinal surface but rapidly spread out radially with increasing distance

from the optic nerve head. Like in adult, experiments concerning the order of retinal axons in the optic nerve in developing monkey (Williams and Rakic, '85) also showed that axons in the optic nerve do not retain a particular set of immediate neighbours and fail to remain in contact with their initial neighbours during their outgrowth through the optic nerve. In adult cat optic nerve, evidence obtained electrophysiologically and anatomically (Horton et al., '79) demonstrated similarly that retinogeniculate axons exist only in a rough retinotopic ordering in the optic nerve for a short distance and quickly become dispersed. At 3 mm from the optic disc, they are no longer confined to a single quadrant of the nerve. This result was confirmed by a more detailed study concerning the retinogeniculate axon order in the optic nerve (Naito, '86). A coarse topographic order was found at the optic nerve head where the axons from different retinal regions maintain the retinal topography in a simplified form according to the trajectory of optic fibres surrounding the optic disc. However, they do not maintain their initial retinotopy along the entire length of the optic nerve, showing a tendency to scatter toward the chiasm.

Although developing retinal axons eventually form precise topographic terminal connections in the brain of these species (Lund, '78), the retinotopic order in the optic nerve is coarse and where it has been studied was found to undergo little change during development. Thus, as in the wallaby, axonal ordering in the mammalian visual pathway was not found to be strictly related to the positions of the axon terminations in the target such as the SC, although it has been concluded from studies on some non-mammalian vertebrates that sequential differentiation of nerve cells could be translated into a well defined spatial organization by simple mechanical principles governing the way in which the growing axons follow their substrate (see above).

SUMMARY

In the wallaby, there is a coarse retinotopic order in the optic nerve during development. The order in the optic nerve may be sufficient to generate initially the coarse retinotopy of collicular innervation from the dorsal and ventral retina, since the grouped dorsal and ventral axons reverse their initial position in the optic nerve and separately enter the SC in the lateral and medial optic tract, respectively. However, it has nothing to do with the final retinotopy of synaptic connections between the optic nerve fibres and collicular neurons. The lack of a causal relationship between retinotopic organization in the optic nerve and in the SC in the wallaby appears consistent with that seen in other mammals. Consequently, a mechanism of pre-ordering of axons in the pathway, suggested as the mechanism for development of retinotopic order in the central target in non-mammalian species, can be ruled out here. The precise order seen in optic pathways in some non-mammalian species is not seen in the mammalian wallaby.

Chapter 6. General conclusion

General plan of formation of the neuronal connections in mammals

Development of the visual projections from the eye to the superior colliculus (SC) has been studied in the tammar wallaby (*Macropus eugenii*). Contralateral retinocollicular projections appear to have two clear developmental stages in the formation of ordering. Retinal axons grow into the superficial layers of the SC in a rough topographical order from the time of their initial ingrowth, the first week after birth. This is followed much later by the formation of neuronal connections, indicated by the arborizations of axon terminals in retinotopically correct positions, the loss of more widely distributed axons and the onset of evoked potentials.

The fact that synaptic transmission cannot be detected until axons abruptly make terminal arborizations in their retinotopically correct position strongly suggests that neuronal specificity of the type originally postulated by Sperry ('44, '63) sets up this retinotopic order in the SC of the wallaby. The topographically correct projection for retinocollicular axons could be achieved by interactions between spatial markers, carried by invading retinal axons and cells in the SC. The evidence of a decrease in size of terminal arborizations after this stage also suggests that impulse activity could contribute to this refinement of the retinocollicular connections in the wallaby, although the initial coarse retinotopic distribution of retinal axons and the initial formation of terminals seems to occur in the absence of impulse activity. Pre-ordering of retinal axons in the visual pathway, suggested as a mechanism for retinotopic order in

central targets in non-mammalian species during development, does not play an important role in the formation of this visual map in the wallaby.

Although not so clearly delineated into two stages as in wallaby some evidence of similar processes may be deduced from work in placental mammals. As in the wallaby, a similar sequence of events and degree of precision in the developing retinocollicular pathway was also demonstrated in cat and ferret (Snider and Chalupa, '93). The straight trajectories of developing axons, with either no branching or only short side branches, followed later by the elaboration of a terminal arborizations are also features of retinocollicular projections in the developing hamster (Schneider et al., '85; Jhaveri et al., '91; Bhide and Frost, '91) and mouse (Sachs et al., '86). Although, it is only in the wallaby that the coincidence of the formation of retinotopically correct terminal arborizations with the onset of functional connections (Mark et al., '93b) has been revealed in the second stage, this may be a general feature of the development of topographic maps in the mammalian brain. For example, this developmental plan is also found in another system, the somatosensory system in the wallaby. Initially, thalamocortical axons are found anatomically to reach the somatosensory cortex by 15 days after birth (Leamey et al., '93). This is followed much later by the onset of functional synapses at 85 days when an evoked potential in response to peripheral stimulation can be recorded eletrophysiologically in the cortex (Waite et al., '91).

Shifting neuronal connections with functional activity, a feature of the developing retinotectal projection of non-mammalian vertebrates in which the earliest retinal fibres to arrive at the tectum have to progressively shift their termination sites on the tectum, does not occur in the wallaby.

Research clues for future studies

The feature of two clearly separated stages in the development of retinocollicular projections of the wallaby, in which the retinal arborizations occur abruptly, at a particular time, in the topographically appropriate area provides opportunities to investigate the underlying cellular and molecular mechanisms involved in the formation of specific neuronal connections. For example, changes in the morphology of cells in the SC could be studied. At a molecular level, there is a distinction between axon guidance in getting to the proper target versus recognition of specific cells within the target tissue.

A number of factors appearing on cell surfaces in targets and distributed in pathways have been described in other species (for review see Bixby and Harris, '91). This includes the cell adhesion molecules such as neuronal cell adhesion molecules (NCAM) and TOP; receptors for growth promoters such as NGF-R, integrins, Ig superfamily molecules and TAG-1; and pathway guidance molecules such as extracellular matrix components, laminin (LN) and fibronectin (FN). It may be possible to examine the changes in level of such molecules in the wallaby, using immunocytochemical techniques. Immunolabelling with the specific antibodies against classes of the molecules likely to be candidates for markers could be used to see if a gradient of molecular components, for example, in the rostrocaudal extent of the SC would be obtained. In addition, the polymerase chain reaction (PCR) techniques and *in situ* hybridization histochemistry could be used to determine whether these known molecules and their receptors are present in either the collicular cells or retinal ganglion cells of the wallaby.

In an effort to identify unknown molecules that could play a role in the rostrocaudal mapping of the retinocollicular projection, chemical immunosuppression techniques (Matthew and Patterson, '83; Matthew and Sandrock, '87; Ou et al., '91) could be used to suppress the production of antigens common to both the first and second stage during development of the retinocollicular projection. A number of monoclonal antibodies, specific for target antigens of interest, that may display a rostrocaudal bias in their binding preferentially to colliculus could then be produced so that rare molecules that are selectively expressed in particular cell types, at the time of axonal arborizations in the retinotopically correct region could be identified in the wallaby. As well, a biochemical assay for protein purification assessed by gel electrophoresis could be also applied to isolate unknown spatial markers.

It would also be interesting to obtain the information, at the electron microscope level, on the onset of synaptic contacts between retinal axons and collicular cells during the same developmental stages. Although the findings from light microscopy and eletrophysiology suggest that synapses would form at the time of onset of function, if they occurred before the onset, a mechanism for exchange of positional information would be provided.

LITERATURE CITED

Baier, H., and F. Bonhoeffer (1992) Axon guidance by gradients of a target-derived component. Science. 255:472-475.

Baker, G.E., and B. E. Reese (1993) Chiasmatic course of temporal retinal axons in the developing ferret. J. Comp. Neurol. 330:95-104.

Banik, N.L., M.J. Blunt, and A.N. Davison (1968) Changes in the osmophilia of myelin and lipid content in the kitten optic nerve. J. Neurochem. 15:471-475.

Bate, C.M. (1976) Pioneer neurons in an insect embryo. Nature. 260:54-56.

Bear, M.F., A. Kleinschmidt, Q. Gu, and W. Singer (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J. Neurosci. 10:909-925.

Beazley, L.D. (1975) Factors determining decussation at the optic chiasma by developing retinotectal fibres in *Xenopus*. Exp. Brain Res. 23:491-504.

Beazley, L.D., and S.A. Dunlop (1983) The evolution of an area centralis and visual streak in the marsupial *Setonix brachyurus*. J. Comp. Neurol. 216:211-231.

Bembridge, B.A. (1956) The problem of myelination in the central nervous system, with special reference to the optic nerve. Trans. Ophthal. Soc. U.K. 76:311-322.

Bentley, D., and H. Keshishian (1982) Pathfinding by peripheral pioneer neurons in grasshoppers. Science. 218:1082-1088.

Bhide, P.G., and D.O. Frost (1991) Stages of growth of hamster retinofugal axons: implication for developing axonal pathways with multiple targets. J. Neurosci. 11:485-504.

Bixby, J.L., and W.A. Harris (1991) Molecular mechanisms of axon growth and guidance. Ann. Rev. Cell Biol. 7:117-159.

Blunt, M.J., F. Baldwin, and C.P. Wendell-Smith (1972) Gliogenesis and myelination in kitten optic nerve. Z. Zellforsch. 124:293-310.

Bodick, N., and C. Levinthal (1980) Growing optic nerve fibers follow neighbours during embryogenesis. Proc. Natl. Acad. Sci. (USA). 77:4374-4378.

Bonhoeffer F., and A. Gierer (1984) How do retinal axons find their targets on the tectum? TINS. 378-381.

Braekevelt, C.R., L.D. Beazley, S.A. Dunlop, and J.E. Darby (1986) Numbers of axons in the optic nerve and of retinal ganglion cells during development in the marsupial *Setonix Brachyurus*. Dev. Brain Res. 25:117-125.

Bunt, S.M. (1982) Retinotopic and temporal organization of the optic nerve and tracts in the adult goldfish. J. Comp. Neurol. 206:209-226.

Bunt, S.M., and R.D. Lund (1982) Optic fibre arrangements in the visual pathways of Long-Evans hooded rats. Soc. Neurosci. Abstr. 8:125.

Bunt, S.M., and T.J. Horder (1983) Evidence for an orderly arrangement of optic axons within the optic nerve of the major nonmammalian vertebrate classes. J. Comp. Neurol. 213:94-114.

Bunt, S.M., R.D. Lund and P.W. Land (1983) Prenatal development of the optic projection in albino and hooded rats. Dev. Brain Res. 6:149-168.

Cajal, S. Ramon y (1892) La rétine des vertèbrés. Translated by Thorpe S.A., M. Glickstein (1972): The structure of the retina. Springfield, Illinois: Thomas.

Cajal, S. Ramon y (1911) Histologie du systeme nerveux de 1'Homme et des Vertèbrés. vol 2 (translated by C. Azoulay). Paris: Maloine.

Campbell, C.B.G., and S.O.E. Ebbesson (1969) The optic system of a teleost: *Holocentrus* re-examined. Brain Behav. Evol. 2:415-430.

Cavalcante, L.A., and C.E. Rocha-Miranda (1978) Postnatal development of retinogeniculate, retino--pretectal and retinotectal projections in the opossum. Brain Res. 146:231-248.

Chalupa, L.M., and B. Lia (1991) The nasotemporal division of retinal ganglion cells with crossed and uncrossed projections in the fetal rhesus monkey. J. Neurosci. 11:191-202.

Chalupa, L.M., and R.W. Williams, and Z. Henderson (1984) Binocular interaction in the fetal cat regulates the size of the ganglion cell population. Neuroscience. 12:1139-1146.

Chase, J. (1982) The evolution of retinal vascularization in mammals. Ophthalmology. 89:1518-1521.

Chelvanayagam, D.K., and L.D. Beazley (1992) Changes in the organization of fibres in the rat optic nerve. Proc. Aust. Neurosci. Soc. 3:170.

Chelvanayagam, D.K., and L.D. Beazley (1994) Optic axons are ordered from early development in the visual pathway of the quokka *Setonix brachyurus*. Proc. Aust. Neurosci. Soc. 5:120.

Chung, S.H., M.J. Keating and T.V.P. Bliss (1974) Functional synaptic relations during the development of the retino-tectal projection in amphibians. Proc. R. Soc. Lond. B. 187:449-459.

Cima, C., and P. Grant (1982) Development of the optic nerve in *Xenopus laevis*. I. Early development and organization. J. Embryol. exp. Morph. 72:225-249.

Clarke, P.G.H., and D. Whitteridge (1976) The projection of the retina, including the "red area," onto the optic tectum of the pigeon. Quart. J. Exp. Physiol. 61:351-358.

Colello, R.J., and R.W. Guiliery (1990) The early development of retinal ganglion cells with uncrossed axons in the mouse: retinal position and axonal course. Development. 108:515-523.

Constantine-Paton, M., and M.I. Law (1978) Eye-specific termination bands in tecta of three-eyed frog. Science. 202:639-641.

Constantine-Paton, M., H.T. Cline, and E. Debski (1990) Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathway. Ann. Rev. Neurosci. 13:129-54.

Cook, J.E. (1983) Tectal paths of regenerating optic axons in the goldfish: Evidence from retrograde labelling with horseradish peroxidase. Exp. Brain Res. 51:433-422.

Coulombre, A.J. (1956) The role of intraocular pressure in the development of the chick eye. I. Control of eye size. J. Exp. Zool. 133:211-225.

Cowey, A, and V.H. Perry (1979) The projection of the temporal retina in rats, studied by retrograde transport of horseradish peroxidase. Exp. Brain Res. 35:457-464.

Cox, E.C., B. Muller, and F. Bonhoeffer (1990) Axonal guidance in the chick visual system: Posterior tectal membranes induce collapse of growth cones from the temporal retina. Neuron. 2:31-37.

Crabtree, J.W. (1989) Prenatal development of retinocollicular projections in the rabbit: an HRP study. J. Comp. Neurol. 286:504-513.

Crespo, D., D.D.M. O'Leary, and W.M. Cowan (1985) Changes in the numbers of optic fibres during late prenatal and postnatal development in the albino rat. Dev. Brain Res. 19:129-134.

Crewther, D.P., J.E. Nelson, and S.G. Crewther (1988) Afferent input for target survival in marsupial visual development. Neurosci. Lett. 86:147-154.

Crossland, W.J., W.M. Cowan, and L.R. Ragers (1975) Studies on the development of the chick optic tectum. IV. An autoradiographic study of the development of retino-tectal connections. Brain Res. 91:1-23.

Cucchiaro, J.B. (1991) Early development of the retinal line of decussation in normal and albino ferrets. J. Comp. Neurol. 312:193-206.

Cunningham, T.J., I.M. Mohler and D.L. Giordano (1982) Naturally occurring neuron death in the ganglion cell layer of the neonatal rat: Morphology and evidence for regional correspondence with neuron death in superior colliculus. Dev. Brain Res. 2:203-215.

Currie, J, and W.M. Cowan (1975) The development of the retino-tectal projection in *Rana pipiens*. Dev. Biol. 46:103-119.

Cusick, C.G., and J.H. Kaas (1982) Retinal projections in adult and newborn grey squirrels. Dev. Brain. Res. 4:275-284.

Cyander, M., and N. Berman (1972) Receptive-field organization of monkey superior colliculus. J. Neurophysiol. 35:187-201.

Davies, J.A., G.M.W. Cook, C.D. Stern and R.J. Keynes (1990) Isolation from chick somites of glycoprotein fraction that causes collapse of dorsal root ganglion growth cones. Neuron. 2:11-20.

Davis, F.A. (1929) The anatomy and histology of the eye and orbit of the rabbit. Trans. Ophthalmol. Soc. U.K. 27:401-441.

De Long, G.R, and A.J. Coulombre (1965) Development of the retinotectal topographic projection in the chick embryo. Exp. Neurol. 13:351-363.

Dowling, J.E. (1970) Organization of vertebrate retinas. Invest. Ophthal. 9:655-680.

Drager, U.C. (1985) Birth dates of retinal ganglion cells giving rise to the crossed and uncrossed optic projections in the mouse. Proc. R. Soc. Lond. B. 224:57-77.

Drager, U.C., and J.F. Olsen (1980) Orgins of crossed and uncrossed retinal projections in pigmented and albino mice. J. Comp. Neurol. 191:383-412.

Drager, U.C., and A. Hofbauer (1984) Antibodies to heavy neurofilament subunit detect a subpopulation of damaged ganglion cells in retina. Nature. 309:624-626.

Dreher, B., and S.R. Robinson (1988) Development of the retinofugal pathway in birds and mammals: evidence for a common 'timetable'. Brain Behav. Evol. 31:369-390.

Dreher, B., R.A. Potts, and M.R. Bennett (1983) Evidence that the early postnatal reduction in the number of rat retinal ganglion cells is due to a wave of ganglion cell death. Neurosci. Lett. 36:255-260.

Dunlop, S.A., and L.D. Beazley (1985) Changing distribution of retinal ganglion cells during area centralis and visual streak formation in the marsupial *Setonix brachyurus*. Dev. Brain Res. 23:81-91.

Dunlop, S.A., and L.D. Beazley (1987) Cell death in the developing retinal ganglion cell layer of the wallaby *Setonix brachyurus*. J. Comp. Neurol. 264:14-23.

Dunlop, S.A., W.A. Longley and L.D. Beazley (1987) Development of the area centralis and visual streak in the grey kangaroo *Macropus fuliginosus*. Vision Res. 27:151-164.

Dunlop, S.A., L.-A. Coleman, A.M. Harman, and L.D. Beazley (1988) Development of the marsupial primary visual pathway. In: The developing marsupial. Tyndale-Biscoe, C.H., and P.A. Janssens (eds). Berlin, Heidelberg, New York. Spring-Verlag.

Easter, S.S., Jr., A.C. Rusoff and P.E. Kish (1981) The growth and organization of the optic nerve and tract in juvenile and adult goldfish. J. Neurosci. 1: 793-811.

Easter, S.S., Jr., and C.A.O. Stuermer (1984) An evaluation of the hypothesis of shifting terminals in goldfish optic tectum. J. Neurosci. 4:1052-1063.

Edwards, M.A., G.E. Schneider, and V.S. Caviness (1986) Development of the crossed retinocollicular projection in the mouse. J. Comp. Neurol. 248:410-421.

Fawcett, I.W. (1976) Fibre organization in the optic nerve of *Xenopus laevis*. J. Physiol. (Lond.) 306:32P.

Fawcett, J.W. (1981) How axons grow down the *Xenopus* optic nerve. J. Embryol. exp. Morph. 65:219-233.

Fawcett, J.W., and D.D.M. O'Leary (1985) The role of electrical activity in the formation of topographic maps in the nervous system. TINS. 201-206.

Fine, A., W.B. Amos, R.M. Durbin and P.A. McNaughton (1988) Confocal microscopy: applications in neurobiology. TINS. 11:346-351.

Fischer, Q.S., and M.A. Kirby (1991) Number and distribution of retinal ganglion cells in anubis baboons (*Papio anubis*). Brain Behav. Evol. 37:189-203.

Flett, D.L. (1986) The topography of retinal projections to the superior colliculus and dorsal lateral geniculate nucleus of a wallaby, the tammar (*Macropus eugenii*). A thesis for Master's degree. The Australian National University.

Flett, D.L., L.R. Marotte, and R.F. Mark (1988) Retinal projections to the superior colliculus and dorsal lateral geniculate nucleus in the tammar wallaby (*Macropus eugenii*): I. Normal topography. J. Comp. Neurol. 271:257-273.

Foster, R.E., B.W. Connors, and S.G. Waxman (1982) Rat optic nerve: Electrophysiological pharmacological and anatomical studies during development. Dev. Brain Res. 3:371-386.

Fraser, S.E. (1980) A differential approach to the patterning of nerve connections. Dev. Biol. 79:453-464.

Fraser, S.E. (1983) Fibre optic mapping of the *Xenopus* visual system: shift in the retinotectal projection during development. Dev. Biol. 95:505-511.

Fraser, C.E., and R.D. Hunt (1980) Retinotectal specificity: Models and experiments in search of a mapping function. Ann. Rev. Neurosci. 3:319-52.

Fraser, S.E., and R.K. Hunt (1986) A physiological measure of shifting connections in the *Rana pipiens* retinotectal system. J. Embryol. exp. Morph. 94:149-161.

Fraser, S.E., and Perkel (1989) Competitive and positional cues in the patterning of nerve connections. J. Neurobiol. 21:51-72.

Freeman, B., and E. Tancred (1978) The number and distribution of ganglion cells in the retina of the brush-tailed possum *Trichosurus vulpecula*. J. Comp. Neurol. 177:557-568.

Freeman, B., and C.R.R. Watson (1978) The optic nerve of the brush-tailed possum, *Trichosurus vulpecula*: Fibre diameter spectrum and conduction latency groups. J. Comp. Neurol. 179:739-752.

Freeman, T.C.B. (1992) The onset of the electrophysiological responses in the superior colliculus of the wallaby (*Macropus eugenii*): A current source density analysis of the evoked potentials seen early in development. A thesis for Honours degree. The Australian National University.

Fries, W., and H. Distel (1983) Large layer VI neurons of monkey striate cortex (Meynert cells) project to the superior colliculus. Proc. R. Soc. Lond. B. 219:53-59.

Fries, W. (1984) Cortical projections to the superior colliculus in the macaque monkey: A retrograde study using horseradish peroxidase. J. Comp. Neurol. 230:55-76.

Frost, D.O., K.-F. So and G.E. Schneider (1979) Postnatal development of retinal projections in Syrian hamsters: a study using autoradiographic and anterograde degeneration techniques. Neuroscience. 4:1649-1677.

Fujisawa, H. (1981) Retinotopic analysis of fiber pathways in the regenerating retinotectal system of the adult newt *Cynops*. Brain Res. 206:27-37.

Fujisawa, H. (1987) Mode of growth of retinal axons within the tectum of *Xenopus* tadpoles, and implications in the ordered neuronal connection between the retina and the tectum. J. Comp. Neurol. 260:127-139.

Fujisawa, H., N. Tani, K. Watanabe, and Y. Ibata (1982) Branching of regenerating retinal axons and preferential section of appropriate branches for specific neuronal connection in the newt. Dev. Biol. 90:43-57.

Fujisawa, H., S. Thanos, and U. Schwarz (1984) Mechanisms in the development of retinotectal projections in the chick embryo studied by surgical deflection of the retinal pathway. Dev. Biol. 102:356-367.

Fukuda, Y., C.-F. Hsiao, H. Sawai, and K. Wakakuwa (1984) Retinotopic organizations of the expanded ipsilateral projection to the rat's superior colliculus; variations along its rostrocaudal axis. Brain Res. 321:390-395.

Garey, L.J., B. Dreher, and S.R. Robinson (1991) The organization of the visual thalamus. In: Vision and visual dysfunction. Cronly-Dillon, J.R. (ed). vol. 3, pp 176-234. Macmillan Press (Scientific and Medical).

Garey, L.J., E.G. Jones, and T.P.S. Powell (1968) Interrelationships of striate and extrastriate cortex with the primary relay sites of the visual pathway. J. Neurol. Neurosurg. Psychiat. 31:135-157.

Gayer, N. S., G.M. Horsburgh, and B.Dreher (1989) Developmental changes in the pattern of retinal projections in pigmented and albino rabbits. Dev. Brain Res. 50:33-54.

Gaze, R.M., M.J. Keating, and S.H. Chung (1974) The evolution of the retinotectal map during development in *Xenopus*. Proc. R. Soc. Lond. B. 185:301-330.

Gaze, R.M, M.J. Keating, A. Ostberg and S-H. Chung (1979) The relationship between retinal and tectal growth in larval *Xenopus*: implications for the development of the retino-tectal projection. J. Embryol. exp. Morph. 53:103-143.

Ghosh, A., and C.J. Shatz (1992) Pathfinding and target selection by developing geniculocortical axons. J. Neurosci. 12:39-55.

Gierer, A. (1987) Directional cues for growing axons forming the retinotectal projection. Development. 101:479-489.

Gilbert, C.D., and J.P. Kelly (1975) The projections of cells in different layers of the cat's visual cortex. J. Comp. Neurol. 163:81-105.

Godement, P., and F. Bonhoeffer (1989) Cross-species recognition of tectal cues by retinal fibres *in vitro*. Development. 106:313-320.

Godement, P., and C.A. Mason (1990) Behaviour of live retinal axons growth cones in the optic chiasm. Soc. Neurosci. Abstr. 16:1125.

Godement, P., J. Salaun, and M. Imbert (1984) Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse. J. Comp. Neurol. 230:552-575.

Godement, P., J. Vanselow, S. Thanos, and F. Bonhoeffer (1987) A study in developing visual systems with a new method of staining neurons and their processes in fixed tissue. Development. 101:697-713.

Godement, P., J. Salaun, and C.A. Mason (1990) Retinal axon pathfinding in the optic chiasma: Divergence of crossed and uncrossed fibres. Neuron. 5:173-186.

Goldberg, M.E., and D.L. Robinson (1978) Visual system: Superior colliculus. In: Handbook of Behavioral Neurobiology. Masterton,R.B. (ed). vol. 1: Sensory integration. pp 119-164. New York, London: Plenum press.

Goldberg, S. (1974) Studies on the mechanics of development of the visual pathway in the chick embryo. Dev. Biol. 36:24-43.

Graham, J. (1982) Some topographical connections of the striate cortex with subcortical structures in the *Macaca fascicularis*. Exp. Brain Res. 47:1-14.

Graybiel, A.M. (1975) Anatomical organization of retinotectal afferents in the cat: An autoradiographic study. Brain Res. 96:1-23.

Graybiel, A.M. (1976) Evidence for banding of the cat's ipsilateral retinotectal connection. Brain Res. 114:318-327.

Gu, Q, M.F. Bear, and W. Singer (1989) Blockade of NMDA-receptor prevents ocularity changes in kitten visual cortex after reversed monocular deprivation. Dev. Brain Res. 47:281-288.

Guillaume, P., L.-C. Wang, C.A. Mason, and P. Godement (1991) Retinal axon-optic chiasm cellular interactions *in vitro*. Soc. Neurosci. Abstr. 17:40.

Guillery, R. (1982) The optic chiasm of the vertebrate brain. Sensory Physiol. 7:39-73.

Guillery, R.W. and V.A. Casagrande (1976) Adaptive synaptic connections formed in the visual pathways in response to congenitally aberrant inputs. Cold Spring Harbour Symp. Quant. Biol. 40:611-617.

Hahm, J-O., R.B. Langdon and M. Sur (1991) Disruption of retinogeniculate afferent segregation by antagonists to NMDA receptors. Nature. 351:568-570.

Haight, J.R., and K.J. Sanderson (1988) Retinal projections in two Australian polyprotodont marsupials: Kowari *Dasyuroides byrnei*, and Fat-tailed dunnart, *Sminthopsis crasicaudata* (Dasyuridae). Brain Behav. Evol. 31:96-110.

Haight, J.R., K.J. Sanderson, L. Neylon, and G.S. Patten (1980) Relationships of the visual cortex in the marsupial brush-tailed possum, *Trichosurus vulpecula:* A horseradish peroxidase and autoradiographic study. J. Anat. 131:387-413.

Harman, A.M., and L.D. Beazley (1986) Development of visual projections in the marsupial, *Setonix brachyurus*. Anat. Embryol. 175:181--188.

Harman, A.M., and L.D. Beazley (1987) Patterns of cytogenesis in the developing retina of the wallaby *Setonix brachyurus*. Anat. Embryol. 177:123-130.

Harman, A.M., and L.D. Beazley (1989) Generation of retinal cells in the wallaby, *Setonix brachyurus* (Quokka). Neuroscience. 28:219-232.

Harman, A.M., J.E. Nelson, S.G. Crewther, and D.P. Crewther (1986) Visual acuity of the northern native cat (*Dasyurus hallucatus*)-Behavioural and anatomical estimates. Behav. Brain Res. 22:211-216.

Harting, J.K., and R.W.Guillery (1976) Organization of retinocollicular pathways in the cat. J. Comp. Neurol. 166:133-144.

Harting, J.K., W.C. Hall, I.T. Diamond, and G.F. Martin (1973) Anterograde degeneration study of the superior colliclus in *Tupaia glis*: Evidence for a subdivision between superficial and deep layers. J. Comp. Neurol. 148:361-386.

Hearn, J.P. (1973) Pituitary inhibition of pregnancy. Nature. 241:207-208.

Hearn, J.P. (1974) The pituitary gland and implantation in the tammar wallaby *Macropus eugenii.* J. Reprod. Fertil. 39:235-241.

Henderson, Z., B.L. Finlay, and K.C. Wikler (1988) Development of ganglion cell topography in ferret retina. J. Neurosci. 8 (4):1194-1205.

Hildebrand, C., and S.G. Waxman (1984) Postnatal differentiation of rat optic nerve fibres: Electron microscopic observations on the development of nodes of Ranvier and axoglial relations. J. Comp. Neurol. 224:25-37.

Ho, R.K., and C.S. Goodman (1982) Peripheral pathways are pioneered by an array of central and peripheral neurons in grasshopper embryos. Nature. 297:404-406.

Hokoc, J.N., and E. Oswaldo-Cruz (1978) Quantitative analysis of the opossum's optic nerve: an electron microscope study. J. Comp. Neurol. 178:773-782.

Holcombe, V., and R.W. Guillery (1984) The organization of retinal maps within the dorsal and ventral lateral geniculate nuclei of the rabbit. J. Comp. Neurol. 225:469-491.

Hollander, H. (1974) The origin of corticotectal projections in the cat. Exp. Brain Res. 21:433-439.

Holt, C.E. (1984) Does timing of axon outgrowth influence initial retinotectal topography in *Xenopus*? J. Neurosci. 4:1130-1152.

Holt, C.E., and W.A. Harris (1983) Order in the initial retinotectal map in Xenopus: a new technique for labelling growing nerve fibres. Nature. 301:150-152.

Honig, M.G., and R.I. Hume (1986) Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures. J. Cell Biol. 103:171-187.

Honig, M.G., and R.I. Hume (1989) DiI and DiO: versatile fluorescent dyes for neuronal labelling and pathway tracing. TINS. 12:333-341.

Horder, T.J., and K.A.C. Martin (1978) Morphogenetics as an alternative to chemospecificity in the formation of nerve connections. In: Cell-Cell Recognition, 32nd Symp. Soc. for Exp. Biol. A.S.G. Curtis (ed). pp 275-358. Cambridge: Cambridge Univ. Press.

Horton, J.C., M.M. Greenwood and D.H. Hubel (1979) Non-retinotopic arrangement of fibres in cat optic nerve. Nature. 282:720-722.

Hubel, D.H., S. LeVay, and T.N. Wiesel (1975) Mode of termination of retinotectal fibres in macaque monkey: An autoradiographic study. Brain Res. 96:25-40.

Hughes, A. (1971) Topographical relationships bewteen the anatomy and physiology of the rabbit visual system. Doc. Ophthalmol. 30:33-159.

Hughes, A. (1974) A comparison of retinal ganglion cell topography in the plains and tree kangaroo. J. Physiol (Lond.). 244:61-63.

Hughes, A. (1975) A quantitative analysis of the cat retinal ganglion cell topography. J. Comp. Neurol. 163:107-128.

Hughes, A. (1977) The topography of vision in mammals of contrasting life-style: Comparative optics and retinal organization. In: Handbook of Sensory Physiology, vol VII/5. The visual system in vertebrates. Crescitelli, F. (ed). pp 613-756. Berlin, Heidelberg, New York: Springer-Verlag.

Hughes, A. (1985) New perspective in retinal organization. In: Progress in retinal research, vol 4. pp 243-313. New York: Pergamon.

Ingle, D. (1973) Evolutionary perspectives on the function of the optic tectum. Brain Behav. Evol. 8:211-237.

Insausti, R., C. Blakemore, and W.M. Cowan (1984) Ganglion cell death during development of ipsilateral retino-collicular projection in golden hamster. Nature. 308:362-365.

Jacobson, M. (1977) Mapping the developing retinotectal projection in frog tadpoles by a double label autoradiographic technique. Brain Res. 127:56-67.

Jeffery, G. (1984) Retinal ganglion cells death and terminal field retraction in the developing rodent visual system. Dev. Brain Res. 13:81-96.

Jeffery, G., and V.H. Perry (1982) Evidence for ganglion cell death during development of the ipsilateral retinal projection in the rat. Dev. Brain Res. 2:176-180.

Jeffery, G., A. Cowey, and H.G.J.M. Kuypers (1981) Bifurcating retinal ganglion cell axons in the rat, demonstrated by retrograde double labelling. Exp. Brain Res. 44:34-40.

Jen, L.S., K.-F. So, and H.H. Woo (1984) An anterograde HRP study of the retinocollicular pathways in normal hamsters and hamsters with one eye enucleated at birth. Brain Res. 294:169-173.

Jhaveri, S., M.A. Edwards, and G.E. Schneider (1983) Relationship of lateral geniculate neuron migration to stages of optic tract growth in the hamster. Soc. Neurosci. Abstr. 9:702.

Jhaveri, S., M.A. Edwards, and G.E. Schneider (1991) Initial stages of retinofugal axon development in the hamster: evidence for two distinct modes of growth. Exp. Brain Res. 87:371-382.

Johnson, G.L. (1901) Contributions to the comparative anatomy of the mammalian eye, chiefly based on ophthalmoscopic examination. Phil. Trans. R. Soc. Lond. B. 194:1-82.

Johnson, J.I., Jr. (1977) Central nervous system of marsupials. In: The biology of marsupials. Hunsaker II, D. (ed). pp159-278. New York, San Francisco and London: Academic press.

Johnston, A.R., and D.J. Gooday (1991) *Xenopus* temporal retinal neurites collapse on contact with glial cells from caudal tectum *in vitro*. Development. 113:409-417.

Kaethner, R.J., and C.A.O. Stuermer (1992) Dynamics of terminal arbor formation and target approach of retinotectal axons in living zebrafish embryos: A time-lapse study of single axons. J. Neurosci. 12: 3257-3271.

Kanaseki, T., and J.M. Sprague (1974) Anatomical organization of pretectal and tectal laminae in the cat. J. Comp. Neurol. 158:319-337.

Kapfhammer, J.P., and J.A. Raper (1987a) Collapse of growth cone structure on contact with specific neurites in culture. J. Neurosci. 7:201-212.

Kapfhammer, J.P., and J.A. Raper (1987b) Interactions between growth cones and neurites growing from different neural tissues in culture. J. Neurosci. 7:1595-1600.

Kawamura, S., J.M. Sprague, and K. Niimi (1974) Corticofugal projection from the visual cortices to the thalamus, pretectum and superior colliculus in the cat. J. Comp. Neurol. 158:339-362.

Kelling, S.T., D.R. Sengelaub, and K.C. Wikler (1989) Differential elasticity of the immature retina: A contribution to the development of the area centralis. Vis. Neurosci. 2:117-120.

Keshishian, H. (1980) The origin and morphogenesis of pioneer neurons in the grasshopper metathoracic leg. Dev. Biol. 16:165-206.

Kirby, M.A., L. Clift-Forsberg, P.D. Wilson, and S.C. Rapisardi (1982) Quantitative analysis of the optic nerve of the North American opossum (*Didelphis virginiana*): An electron microscopic study. J. Comp. Neurol. 211:318-327.

Kirby, M.A., P.D. Wilson, and T.M. Fischer (1988) Development of the optic nerve of the opossum. Dev. Brain Res. 44:37-48.

Kirsch, J.A.W., and J.H. Calaby (1977) The species of living marsupials: an annotated list. In: The biology of marsupials. Stonehouse, B. and D. Gilmore (eds). pp9-26. London: Macmillan press; Baltimore: University Park Press.

Kleinschmidt, A., M.F. Bear, and W. Singer (1987) Blockade of "NMDA" receptors disrupts experience-dependent plasticity of kitten striate cortex. Science. 238:355-358.

Kollros, J.J. (1953) The development of the optic lobes in the frog. I. The effects of unilateral enucleation in embryonic stages. J. Exp. Zool. 123:153-187.

Kruger, L. (1970) The topography of the visual projection to the mesencephalon: a comparative survey. Brain Behav. Evol. 3:169-177.

Kunzle, H.K., and K. Akert (1977) Efferent connections of cortical area 8 (frontal eye field) in *Macaca fascicularis*: A reinvestigation using the autoradiographic technique. J. Comp. Neurol. 173:147-164.

Lam, K., A.J. Sefton, and M.R. Bennett (1982) Loss of axons from the optic nerve of the rat during early postnatal development. Dev. Brain Res. 3:487-491.

Land, P.W., and R.D. Lund (1979) Development of the rat's uncrossed retinotectal pathway and its relation to plasticity studies. Science. 205:698-700.

Lane, R.H., J.M. Allman, J.H. Daas, and F.M. Miezin (1973) The visuotopic organization of the superior colliculus of the owl monkey (*Aotus trivirgatus*) and the bush baby (*Galago senegalensis*). Brain Res. 60:335-349.

Lashley, K.S. (1934) The mechanism of vision. VII. The projection of the retina upon the primary optic centres in the rat. J. Comp. Neurol. 59:341-373.

LaVail, J.H, and W.M. Cowan (1971a) The development of the chick optic tectum. II. Autoradiographic studies. Brain Res. 28:421-441.

LaVail, J.H., and W.M. Cowan (1971b) The development of the chick optic tectum. I. Normal morphology and cytoarchitectonic development. Brain Res. 28:391-419.

Leamey, C.A., P.M.E. Waite, L.R. Marotte, and R.F. Mark (1993) The development of the trigeminal pathway from periphery to cortex in the wallaby (*Macropus eugenii*). Proc. Aust. Neurosci. Soc. 4:103.

Leventhal, A.G., J.D. Schall, S.J. Ault, J.M. Provis, and D.J. Vitek (1988) Class-specific cell death shapes the distribution and pattern of central projection of cat retinal ganglion cells. J. Neurosci. 8:2011-2027.

Lia, B., R.W. Williams, and L.M.Chalupa (1987) Formation of retinal ganglion cell topography during prenatal development. Science. 236:848-851.

Linden, R., and V.H. Perry (1982) Ganglion cell death within the developing retina: A regulatory role for retinal dendrites? Neuroscience. 7:2813-2827.

Lund, R.D (1965) Uncrossed visual pathway of hooded and albino rats. Science. 149:1506-1507.

Lund, R.D. (1972) Synaptic patterns in the superficial layers of the superior colliculus of the monkey. *Macaca mulatta*. Exp. Brain Res. 15:194-211.

Lund, R.D. (1978) Development and plasticity of the brain. New York: Oxford Univ. Press.

Lund, R.D., and H. Bunt (1976) Prenatal development of central optic pathways in albino rats. J. Comp. Neurol. 165:247--264.

Lund, R.D., J.S. Lund, and R.P. Wise (1974) The organization of the retinal projection to the dorsal lateral geniculate nucleus in pigmented and albino rats. J. Comp. Neurol. 158:383-404.

Mann, I. (1964) The development of the eye. London: Br. Med. Assoc.

Mark, R.F., and L.R. Marotte (1992) Australian marsupials as models for the developing mammalian visual system. TINS. 15 (2):51-57.

Mark, R.F., A.C. James, and X.-M. Sheng (1993a) Geometry of the representation of the visual field on the superior colliculus of the wallaby (*Macropus eugenii*). I. Normal projection. J. Comp. Neurol. 330:303-314.

Mark, R.F., T.C.B. Freeman, Y. Ding, and L.R. Marotte (1993b) Two stages in the development of a mammalian retinocollicular projection. NeuroReport. 5:117-120.

Marotte, L.R. (1990) Development of retinotopy in projections from the eye to the dorsal lateral geniculate nucleus and superior colliculus of the wallaby (*Macropus eugenii*). J. Comp. Neurol. 293:524-539.

Marotte, L.R. (1993) Location of retinal ganglion cells contributing to the early imprecision in the retinotopic order of the developing projection to the superior colliculus of the wallaby (*Macropus eugenii*). J. Comp. Neurol. 331:1-13.

Martin, P.R., A. J. Sefton, and B. Dreher (1983) The retinal location and fate of ganglion cells which project to the ipsilateral superior colliculus in neonatal albino and hooded rats. Neurosci. Lett. 41:219-226.

Mason, C.A., J.P. Misson, R. Blazeski, and P. Godement (1990) Retinal axon navigation in the mouse optic chiasm: Midline cues and cell-cell relations. Soc. Neurosci. Abstr. 16:1125.

Mastronarde, D.N., M.A. Thibeault, and M.M. Dubin (1984) Non-uniform postnatal growth of the cat retina. J. Comp. Neurol. 228:598-608.

Matthew, W.D., and P.H. Patterson (1983) The production of a monoclonal antibody that blocks the action of a neurite outgrowth-promoting factor. Cold Spring Harbor Symp. Quant. Biol. 48:625-631.

Matthew, W.D., and A.W. Sandrock (1987) Cyclophosphamide treatment used to manipulate the immune response for the production of monoclonal antibodies. J. Immunol. Methods. 100:73-82.

McCall, M.J., S.R. Robinson, and B. Dreher (1987) Differential retina growth appears to be the primary factor producing the ganglion cell density gradient in the rat. Neurosci. Lett. 79:78-84.

McIlwain, J.T. (1977) Topographic organization and convergence in corticotectal projections from areas 17, 18 and 19 in the cat. J. Neurophysiol. 40:189-198.

McLoon, S. (1982) Alterations in precision of the crossed retinotectal projection during chick development. Science. 215:1418-1420.

McLoon, S.C. (1985) Evidence for shifting connections during development of the chick retinotectal projection. J. Neurosci. 5:2570--2580.

Merchant, J.C. (1979) The effect of pregnancy on the interval between one oestrus and the next in the tammar wallaby, *Macropus eugenii*. J. Reprod. Fertil. 56:459-463.

Mesulam, M.-M. (1982) Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways-Axonal transport, enzyme histochemistry and light microscopic analysis. In: Tracing neural connections. Mesulam, M.-M. (ed). pp 124-131. New York: John Wiley and Sons.

Meyer, R.L. (1983) Tetrodotoxin inhibits the formation of refined retinotopography in goldfish. Dev. Brain Res. 6:293-298.

Miller, K.D., and M.P. Stryker (1990) Development of ocular dominance columns: mechanisms and models. In Connectionist modeling and brain function: The developing interface. S.J. Hanson and C.R. Olson, eds. (Cambridge, Massachusetts: MIT Press), pp 255-305.

Molotchnikoff, S., and S.K. Itaya (1993) Functional development of the neonatal rat retinotectal pathway. Dev. Brain Res. 72:300-304.

Moore, C.L., R. Kalil, and W. Richards (1976) Development of myelination in optic tract of the cat. J. Comp. Neurol. 165:125-136.

Morest, D.K. (1970) The pattern of neurogenesis in the retina of the rat. Z. Anat. Entwicklungsgesch. 131:45-67.

Naito, J. (1986) Course of retinogeniculate projection fibres in the cat optic nerve. J. Comp. Neurol. 254:376-387.

Naito, J. (1989) Retinogeniculate projection fibres in the monkey optic nerve: a demonstration of the fiber pathways by retrograde axonal transport of WGA-HRP. J. Comp. Neurol. 284:174-186.

Nakamura, H., and D.D.M. O'Leary (1989) Inaccuracies in initial growth and arborization of chick retinotectal axons followed by course corrections and axon remodelling to develop topographic order. J. Neurosci. 9:3776-3795.

Ng, A.Y.K., and J. Stone (1982) The optic nerve of the cat: Appearance and loss of axons during normal development. Dev. Brain Res. 5:263-271.

Norris, W.E., C.D. Stern and R.J. Keynes (1989) Molecular differences between the rostral and caudal halves of the sclerotome in the chick embryo. Development. 105:541-548.

O'Leary, D.D.M., J.W. Fawcett, and W.M. Cowan (1986) Topographic targeting errors in the retinocollicular projection and their elimination by selective ganglion cell death. J. Neurosci. 6:3692-3705.

O'Rourke, N.A., and S.E. Fraser (1986) Dynamic aspects of retinotectal map formation as revealed by a vital-dye fiber-tracing technique. Dev. Biol. 114:265-276.

O'Rourke, N.A., and S.E. Fraser (1990) Dynamic changes in optic fiber terminal arbors lead to retinotopic map formation: An *in vivo* confocal microscopic study. Neuron. 5:159-171.

Olavarria, J., and R. Van Sluyters (1982) The projection from striate and extrastriate cortical areas to the superior colliculus in the rat. Brain Res. 242:332-336.

Olszewski, J., and D. Baxter (1954) Cytoarchitecture of the human brain stem. Basel: Karger.

Ostrach, L.H., M.A. Kirby, and L.M. Chalupa (1986) Topographic organization of retinocollicular projection in the fetal cat. Soc. Neurosci. Abstr. 12:119.

Ou, S. K.-H., C. McDonald, and P.H. Patterson (1991) Comparison of two techniques for targeting the production of monoclonal antibodies against particular antigens. J. Immunol. Methods. 145:111-118.

Oyster, C.W., E.S. Takahashi, and D.C. Hurst (1981) Density, soma size and regional distribution of rabbit retinal ganglion cells. J. Neurosci. 1:1331-1346.

Pearson, L.J., K.J. Sanderson and R.T. Wells (1976) Retinal projections in the ringtailed possum *Pseudocheirus peregrinus.* J. Comp. Neurol. 170:227-240.

Perry, V.H. (1981) Evidence for an amacrine cell system in the ganglion cell layer of the rat retina. Neuroscience. 6:931-944.

Perry, V.H., and A. Cowey (1985) The ganglion cell and cone distributions in the monkey's retina: Implications for central magnification factors. Vis. Res. 25:1795-1810.

Perry, V.H., Z. Henderson, and R. Linden (1983) Postnatal changes in retinal ganglion cell and optic axon populations in the pigmented rat. J. Comp. Neurol. 219:356-368.

Potts, R.A., B. Dreher, and M.R. Bennett (1982) The loss of ganglion cells in the developing retina of the rat. Dev. Brain Res. 3:481-486.

Provis, J.M., F.A. Billson, and P. Russell (1983) Ganglion cell topography in human fetal retinae. Invest. Ophthalmol. Vis. Sci. 24:1316-1320.

Provis, J.M., D. van Driel, F.A. Billson, and P. Russell (1985) Human fetal optic nerve: overproduction and elimination of retinal axons during development. J. Comp. Neurol. 238:92-100.

Rager, G., and B. von Oeynhausen (1979) Ingrowth and ramification of retinal fibers in the developing optic tectum of the chick embryo. Exp. Brain Res. 35:213-227.

Rager, G., and U. Rager (1978) Systems--matching by degeneration. I. A quantitative electron microscopic study of the generation and degeneration of retinal ganglion cells in the chicken. Exp. Brain Res. 33:65-78.

Rager, G.H. (1980a) Specificity of nerve connections by unspecific mechanisms? TINS. 3:43-44.

Rager, G.H. (1980b) Development of the retinotectal projection in the chicken. Adv. Anat. Embryol. Cell Biol. Springer-Verlag, Berlin. pp 44-55.

Rakic, P. (1977) Prenatal development of the visual system in rhesus monkey. Phil. Trans. R. Soc. Lond. B. 278:245-260.

Rakic, P., and K.P. Riley (1983) Overproduction and eliminating of retinal axons in the fetal rhesus monkey. Science. 219:1441-1444.

Rapaport, D.H., and J. Stone (1983) The topography of cytogenesis in the developing retina of the cat. J. Neurosci. 3:1824-1834.

Rapaport, D.H., P.D. Wilson, and M.H. Rowe (1981) The distribution of ganglion cells in the retina of the North American opossum (*Didelphis virginiana*). J. Comp. Neurol. 199:465-480.

Reese, B.E., and A. Cowey (1986) Large retinal ganglion cells in the rat: their distribution and laterality of projection. Exp. Brain Res. 61:375-385.

Reese, B.E., and R.J. Colello (1992) Neurogenesis in the retinal ganglion cell layer of the rat. Neuroscience. 46:419-429.

Reese, B.E., R.W. Guillery, and C. Mallarino (1992) Time of ganglion cell genesis in relation to the chiasmatic pathway choice of retinofugal axons. J. Comp. Neurol. 324:336-342.

Reh, T.A., and M.C. Constantine-Paton (1984) Retinal ganglion cell terminals change their projection sites during larval development of *Rana pipiens*. J. Neurosci. 4:442-457.

Reh, T.A., E. Pitts, and M. Constantine-Paton (1983) The organization of the fibers in the optic nerve of normal and tectum-less *Rana pipiens*. J. Comp. Neurol. 218:282-296.

Renfree, M.B. (1979) Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial. Nature. 278:549-511.

Renfree, M.B., A.B. Holt, S.W. Green, J.P. Carr, and D.B. Cheek (1982) Ontogeny of the brain in a marsupial (*Macropus eugenii*) throughout pouch life I. Brain growth and structure. Brain Behav. Evol. 20:57-71.

Renfree, M.B., and C.H. Tyndale-Biscoe (1978) Manipulation of marsupial embryos and pouch young. In: Methods in mammalian reproduction. Daniel, J.C. (ed). London, New York: Academic Press.

Rhoades, R.W., R.D. Mooney, and S.E. Fish (1991) Retinotopic and visuotopic representations in the mammalian superior colliculus. In: Vision and visual dysfunction. Cronly-Dillon, J.R. (ed). vol. 3, pp 150-175. Macmilan press (Scientific and Medical).

Robinson, S.R. (1991) Development of the mammalian retina. In: Vision and visual dysfunction. Cronly-Dillon, J.R. (ed). vol. 3, pp 69-128. Macmillan press (Scientific and Medical).

Robinson, S.R., and B. Dreher (1990) The visual pathways of eutherian mammals and marsupials develop according to a common timetable. Brain Behav. Evol. 36:177-195.

Robinson, S.R., B. Dreher, and M.J. McCall (1989) Nonuniform retinal expansion during the formation of the rabbit's visual streak: Implications for the ontogeny of mammalian retinal topography. Vis. Neurosci. 2:201-219.

Robinson, S.R., G.M. Horsburgh, B. Dreher, and M.J. McCall (1987) Changes in the numbers of retinal ganglion cells and optic nerve axons in the developing rabbit. Dev. Brain Res. 35:161-174.

Rodieck, R.W. (1973) The vertebrate retina: Principles of structure and function. San Francisco: Freeman.

Rolls, E.T., and A. Cowey (1970) Topography of the retina and striate cortex and its relationship to visual acuity in rhesus monkeys and squirrel monkeys. Exp. Brain Res. 10:298-310.

Romer, A.S., and T.S. Parsons (1977) The vertebrate body. Philadelphia, London, Toronto. W.B. Saunders Company.

Royce, G.J., J.P. Ward, and J.K. Harting (1976) Retinofugal pathways in two marsupials. J. Comp. Neurol. 170:391-414.

Rusoff, A.C., and S.S. Easter, Jr. (1980) Order in the optic nerve of goldfish. Science. 208:311-312.

Sachs, G., M. Jacobson, and V.S. Caviness, JR. (1986) Postnatal changes in arborization patterns of murine retinocollicular axons. J. Comp. Neurol. 246:395-408.

Sakaguchi, D.S., and R.K. Murphey (1985) Map formation in the developing *Xenopus* retinotectal system: An examination of ganglion cell terminal arborizations. J. Neurosci. 5:3228-3245.

Sanderson, K.J., and L.J. Pearson (1977) Retinal projections in the native cat, *Dasyurus viverrinus.* J. Comp. Neurol. 174:347-358.

Sanderson, K.J., L.J. Pearson, and P.G. Dixon (1978) Altered retinal projections in brushtailed possum, *Trichosurus vulpecula,* following removal of one eye. J. Comp. Neurol. 180:841-868.

Sanderson, K.J., L.J. Pearson, and J.R. Haight (1979) Retinal projections in the Tasmanian devil, *Sarcophilus harrisii.* J. Comp. Neurol. 188:335-346.

Sanderson, K.J., P.G. Dixon, and L.J. Pearson (1982) Postnatal development of retinal projections in the brushtailed possum, *Trichosurus vulpecula.* Dev. Brain Res. 5:161-180.

Scalia, F., and V. Arango (1983) The anti-retinotopic organization of the frog's optic nerve. Brain Res. 266:121-126.

Schiller, P. (1984) The superior colliculus and visual function. In: Handbook of physiology, Section I: The nervous system. Sensory Processes, Part I, vol. 3, chap. 11. Brookhart, J.M., and V.B. Mountcastle (eds). pp 457-506. Bethesda: American Physiological Society.

Schmidt, J.T., and L.E. Eisele (1985) Stroboscopic illumination and dark rearing block the sharpening of the regenerated retinotectal map in goldfish. Neuroscience. 14:535-546.

Schmidt, J.T., and B.B. Tieman (1989) Activity, growth cones and the selectivity of visual connections. Comments Dev. Biol. 1:11-28.

Schmidt, J.T. (1990) Long term potentiation and activity-dependent retinotopic sharpening in the regenerating retinotectal projection of goldfish: common sensitive period and sensitivity to NMDA blockers. J. Neurosci. 10:233-246.

Schmid, K.L., L.M. Schmid, C.F. Wildsoet, and J.D. Pettigrew (1992) Retinal topography in the Koala (*Phascolarctos cinereus*). Brain Behav. Evol. 39:8-16.

Schneider, G., S. Jhaveri, M.A. Edwards, and K.F. So (1985) Regeneration, re-routing, and redistribution of axons after early lesions: changes with age, and functional impact. In: Recent achievements in restorative neurology 1: Upper motor neuron functions and dysfunction. Dimitrjijevic, M., and J.C. Eccles (eds). pp 291-310. Basel: Karger.

Scholes, J.H. (1979) Nerve fibre topography in the retinal projection to the tectum. Nature. 278:620-624.

Scott, T.M. (1973) Degeneration of optic nerve terminals in the frog tectum. J. Anat. 114:261-269.

Sefton, A.J., and K. Lam (1984) Quantitative and morphological studies on developing optic axons in normal and enucleated albino rats. Exp. Brain Res. 57:107-117.

Sefton, A.J., A. Mackay-Sim, L.A. Baur, and L.J. Cottee (1981) Cortical projections to visual centres in the rat: An HRP study. Brain Res. 215:1-13.

Segal, R.L., and R.M. Beckstead (1984) The lateral suprasylvian corticotectal projection in cats. J. Comp. Neurol. 225:259-275.

Segal, R.L., R.M. Beckstead, K. Kersey, and S.B. Edwards (1983) The prefrontal corticotectal projection in the cat. Exp. Brain Res. 51:423-432.

Sengelaub, D.R., and B.L. Finlay (1982) Cell death in the mammalian visual system during normal development: I. Retinal ganglion cells. J. Comp. Neurol. 204:311-317.

Sengelaub, D.R., R.P. Dolan, and B.L. Finlay (1986) Cell generation, death, and retinal growth in the development of the hamster retinal ganglion cell layer. J. Comp. Neurol. 246:527-543.

Shatz, C.J. (1990a) Competitive interactions between retinal ganglion cells during prenatal development. J. Neurobiol. 197-211.

Shatz, C.J. (1990b) Impulse activity and the patterning of connections during CNS development. Neuron. 5:745-756.

Sheng, X.M., L.R. Marotte, and R.F. Mark (1990) Development of connections to and from the visual cortex in the wallaby (*Macropus eugeii*). J. Comp. Neurol. 300:196-210.

Silver, J. (1984) Studies on the factors that govern directionality of axonal growth in the embryonic optic nerve and at the chiasm of mice. J. Comp. Neurol. 223:238-251.

Silver, J., and J. Sapiro (1981) Axonal guidance during development of the optic nerve: the role of pigmented epithelia and other extrinsic factors. J. Comp. Neurol. 202:521-538.

Siminoff, R., H.O. Schwassmann, and L. Kruger (1966) An electrophysiological study of the visual projection to the superior colliculus of the rat. J. Comp. Neurol. 127:435-444.

Simon, D.K., and D.D.M. O'Leary (1990) Limited topographic specificity in the targeting and branching of mammalian retinal axons. Dev. Biol. 137:125-134.

Simon, D.K., and D.D.M. O'Leary (1991) Relationship of retinotopic ordering of axons in the optic pathway to the formation of visual maps in central targets. J. Comp. Neurol. 307:393-404.

Simon, D.K., and D.D.M. O'Leary (1992a) Development of topographic order in the mammalian retinocollicular projection. J. Neurosci. 12:1212-1232.

Simon, D.K., and D.D.M. O'Leary (1992b) Influence of position along the medial-lateral axis of the superior colliculus on the topographic targeting and survival of retinal axons. Dev. Brain Res. 69:167-172.

Simon, D.K., and D.D.M. O'Leary (1992c) Responses of retinal axons *in vivo* and *in vitro* to position-encoding molecules in the embryonic superior colliculus. Neuron. 9:977-989.

Simon, D.K., G.T. Prusky, D.D.M. O'Leary, and M. Constantine-Paton (1992) *N*-Methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. Proc. Natl. Acad. Sci. (USA). 89:10593-10597.

Snider, C.J., and L.M. Chalupa (1993) Specificity of the retinocollicular pathway in developing cat and ferret. Soc. Neurosci. Abstr. 19:191.

Sparks, D.L. (1986) Translations of sensory signals into commands for control of saccadic eye movements: Role of primate superior colliculus. Physiol. Reviews. 66 (1):118-171.

Sperry, R.W. (1944) Optic nerve regeneration with return of vision in anurans. J. Neurophysiol. 7:57-69.

Sperry, R.W. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc. Natl. Acad. Sci. (USA). 50:703-710.

Spira, A.W., and L.R. Marotte (1989) Histological and electron microscopic milestones in the development of the retina of a marsupial wallaby, *Macropus eugenii.* Anat. Embryol. 179:571-585.

Sprague, J.M., G. Berlucchi, and G. Rizzolatti (1973) The role of the superior colliculus and pretectum in vision and visually guided behaviour. In: Handbook of sensory physiology. R. Jung (ed). vol. vii/3B. pp 27-101. Berlin, Heidelberg, New York: Springer-Verlag.

Sretavan, D.W. (1990) Specific routing of retinal ganglion cell axons at the mammalian optic chiasm during embryonic development. J. Neurosci. 10:1995-2007.

Sretavan, D.W., and C.J. Shatz (1984) Prenatal development of individual retinogeniculate axons during the period of segregation. Nature. 308:845-848.

Sretavan, D.W., and C.J. Shatz (1986) Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers within the cat's lateral geniculate nucleus. J. Neurosci. 6:234-251.

Sretavan, D.W., and L.F. Reichardt (1993) Time-lapse video analysis of retinal ganglion cell axon pathfinding at the mammalian optic chiasm: growth cone guidance using intrinsic chiasm cues. Neuron. 10:761-777.

Stahl, B., B. Muller, Y. von Boxgerg, E.C. Cox, and F. Bonhoeffer (1990) Biochemical characterization of a putative axonal guidance molecule of the chick visual system. Neuron. 5:735-743.

Stein, B.E. (1981) Organization of the rodent superior colliculus: some comparisons with other mammals. Behav. Brain Res. 3:175-188.

Stone, J. (1965) A quantitative analysis of the distribution of ganglion cells in the cat's retina. J. Comp. Neurol. 124:337-352.

Stone, J., J. Leicester, and S.M. Sherman (1973) The naso-temporal division of the monkey retina. J. Comp. Neurol. 150:33-348.

Stone, J., and E. Johnston (1981) The topography of primate retina: A study of the human, bushbaby, and new- and old-world monkeys. J. Comp. Neurol. 196:205-223.

Stone, J., D.H. Rapaport, R.W. Williams, and L.M. Chalupa (1982) Uniformity of cell distribution in the ganglion cell layer of prenatal cat retina: Implications for mechanisms of retinal development. Dev. Brain Res. 2:231-242.

Stone, J. (1983) Parallel processing in the visual system: the classification of retinal ganglion cells and its impact on the neurobiology of vision. New York: Plenum.

Stone, J., and Z. Dreher (1987) Relationship between astrocytes, ganglion cells and vasculature of the retina. J. Comp. Neurol. 255:35-49.

Stonehouse, B., and D. Gilmore (1977) The biology of marsupials. London: Macmillan Press; Baltimore: University Park Press.

Straznicky, K., and R.M. Gaze (1972) The development of the tectum in *Xenopus laevis*: an autoradiographic study. J. Embryol. exp. Morph. 28:87-115.

Stryker, M.P., and W. A. Harris (1986) Binocular impulse blokade prevents the formation of ocular dominance columns in cat visual cortex. J. Neurosci. 6:2177-2133.

Stuermer, C.A.O. (1988a) Retinotopic organization of the developing retinotectal projection in the zebrafish embryo. J. Neurosci. 8:4513-4530.

Stuermer, C.A.O. (1988b) Trajectories of regenerating retinal axons in the goldfish tectum: I. A comparison of normal and regenerated axons at late regenerated stages. J. Comp. Neurol. 267:56-68.

Stuermer, C.A.O. (1988c) Trajectories of regenerating retinal axons in the goldfish tectum: II. Exploratory branches and growth cones on axons at early regeneration stages. J. Comp. Neurol. 267:69-91.

Stuermer, C.A.O., and P.A. Raymond (1989) Developing retinotectal projection in larval goldfish. J. Comp. Neurol. 281:630-640.

Stuermer, C.A.O. (1990) Target recognition and dynamics of axonal growth in the retinotectal system of fish. Neurosci. Res. Suppl. 13:S1-S10.

Szekely, G. (1973) Anatomy and synaptology of the optic tectum. In: Handbook of sensory physiology. R. Jung (ed). vol. vii/3B. pp 1-26. Berlin, Heidelberg, New York: Springer-Verlag.

Tancred, E., (1981) The distribution and sizes of ganglion cells in the retinas of five Australian marsupials. J. Comp. Neurol. 196:585-603.

Tay, D., K.-F. So, L.S. Jen, and K.C. Lau (1986) The postnatal development of the optic nerve in hamsters: an electron microscopic study. Dev. Brain Res. 30:268-273.

Taylor, J.S.H. (1987) Fibre organization and reorganization in the retinotectal projection of *Xenopus*. Development. 99: 393-410.

Thanos, S., and F. Bonhoeffer (1983) Investigations on the development and topographic order of retinotectal axons: Anterograde and retrograde staining of axons and perikarya with rhodamine *in vivo*. J. Comp. Neurol. 249:430.

Thanos, S., and F. Bonhoeffer (1987) Axonal arborization in the developing chick retinotectal system. J. Comp. Neurol. 261:155-164.

Thompson, I.D., and J.E. Morgan (1993) The development of retinal ganglion cell decussation patterns in postnatal pigmented and albino ferrets. European J. Neurosci. 5:341-356.

Tiao, Y-C., and C. Blakemore (1976) Regional specialization in the golden hamster's retina. J. Comp. Neurol. 168:439-458.

Trisler, D. (1990) Cell recognition and pattern formation in the developing nervous system. J. Exp. Biol. 153:11-27.

Trisler, D., and F. Collins (1987) Corresponding spatial gradients of TOP molecules in the developing retina and optic tectum. Science. 237:1208-1209.

Trisler, D., J. Bekenstein, and M.P. Daniels (1986) Antibody to a molecular marker of cell position inhibits synapse formation in the retina. Proc. Natl. Acad. Sci. (USA). 83:4194-4198.

Tyndale-Biscoe, C.H. (1973) Life of marsupials. London: Edward Arnold.

Tyndale-Biscoe, C.H., and L.A. Hinds (1984) Seasonal patterns of circulating progesterone and prolactin and response to bromocriptine in the female tammar wallaby *Macropus eugenii.* Gen. Comp. Endocrinol. 53:58-68.

Tyndale-Biscoe, C.H., and P.A. Janssens (1988) The developing marsupial. Berlin, Heidelberg, New York: Springer-Verlag.

Tyndale-Biscoe, C.H., J.P. Hearn, and M.B. Renfree (1974) Control of reproduction in macropodid marsupials. J. Endocrinol. 63:589-614.

Udin, S.B., and J.W. Fawcett (1988) Formation of topographic maps. Ann. Rev. Neurosci. 11:289-327.

Updyke, B.V. (1977) Topographic organization of the projections from cortical areas 17, 18 and 19 onto the thalamus, pretectum and superior colliculus in the cat. J. Comp. Neurol. 173:81-122.

Van Buren, J.M. (1963) The retinal ganglion cell layer. Springfield, IL: Charles C. Thomas.

Vaney, D.I., L. Peichl, and B.B. Boycott (1981) Matching populations of amacrine cells in the inner nuclear and ganglion cell layers of the rabbit retina. J. Comp. Neurol. 199:373-391.

Vielmetter, J., and C.A.O. Stuermer (1989) Goldfish retinal axons respond to position-specific properties of tectal cell membranes *in vitro.* Neuron. 2:1331-1339.

Viktorov, I.V. (1966) Neuronal structure of anterior corporal bigemina in insectivora and rodents. Arkh. Anat. Gistol. Embryol. 51:82-89.

Volchan, E., L. de Gonzaga Gawryszewski, and C.E. Rocha-Miranda (1982) Visuotopic organization of the superior colliculus of the opossum. Exp. Brain Res. 46:263-268.

Waite, P.M.E., L.R. Marotte, and R.F. Mark (1991) Development of whisker representation in the cortex of the tammar wallaby *Macropus eugenii*. Dev. Brain Res. 58:35-41.

Walter, J., B. Kern-Veits, J. Huf, and B. Stolze (1987a) Recognition of position-specific properties of tectal cell memberanes by retinal axons *in vitro*. Development. 101:685-696.

Walter, J., S. Henke-Fahle, and F. Bonhoeffer (1987b) Avoidance of posterior tectal membranes by temporal retinal axons. Development. 101:909-913.

Walter, J., T.E. Allsopp, and F. Bonhoeffer (1990) A common denominator of growth cone guidance and collapse? TINS. 13 (11):447-452.

White, J.G., W.B. Amos, and M. Fordham (1987) An evaluation of confocal versus conventional imaging of biological structures by fluorescence light microscopy. J. Cell Biol. 105:41-48.

Whitelaw, V.A., and J.D. Cowan (1981) Specificity and plasticity of retinotectal connections: a computational model. J. Neurosci. 1:1369-1387.

Wikler, K.C., J.I. Raabe, and B.L. Finlay (1985) Temporal retina is preferentially represented in the early retinotectal projection in the hamster. Dev. Brain Res. 21:152-155.

Williams, R.W., and L.M. Chalupa (1982) Prenatal development of retinocollicular projections in the cat: an anterograde tracer transport study. J. Neurosci. 2:604-622.

Williams, R.W., M.J. Bastiani, and L.M. Chalupa (1983) Loss of axons in the cat optic nerve following fetal unilateral ennucleation: An electron microscopic analysis. J. Neurosci. 3:133-144.

Williams, R.W., and P. Rakic (1985) Dispersion of growing axons within the optic nerve of the embryonic monkey. Proc. Natl. Acad. Sci. (USA). 82:3906-3910.

Williams, R.W., M.J. Bastiani, B. Lia, and L.M. Chalupa (1986) Growth cones, dying axons, and developmental fluctuations in the fiber population of the cat's optic nerve. J. Comp. Neurol. 246:32-69.

Willshaw, D.J., and C. von der Malsburg (1976) How patterned neural connections can be set up by self-organization. Proc. R. Soc. Lond. B. 194:431-445.

Willshaw, D.J., and C. von der Malsburg (1979) A marker induction mechanism for the establishment of ordered neural mappings: Its application to the retinotectal problem. Philos. Trans. R. Soc. B 287:203-243.

Wise, R.P., and R.D. Lund (1976) The retina and central projections of heterochromic rats. Exp. Neurol. 51:68-71.

Wong, R.O.L., and A. Hughes (1987) The morphology, number and distribution of a large population of confirmed displaced amacrine cells in the adult cat retina. J. Comp. Neurol. 255:159-177.

Wong, R.O.L., J. Wye-Dvorak, and G.H. Henry (1986) Morphology and distribution of neurons in the retinal ganglion cell layer of the adult tammar wallaby-*Macropus eugenii*. J. Comp. Neurol. 253:1-12.

Wye-Dvorak, J. (1984) Postnatal development of primary visual projections in tammar wallaby (*Macropus eugenii*). J. Comp. Neurol. 228:491-508.

Yaginuma, H., S. Homma, R. Kunzi, and R.W. Oppenheim (1991) Pathfinding by growth cones of commissural interneurons in the chick embryo spinal cord: a light and electron microscope study. J. Comp. Neurol. 334:78-102.

Yhip, J.P.A., and M.A. Kirby (1990) Topographic organization of the retinocollicular projection in the neonatal rat. Vis. Neurosci. 4:313-329.

Poster 15

RETINAL PROJECTIONS IN THE QUOKKA (*SETONIX BRACHYURUS*) AFTER EARLY TECTAL ABLATION.

[1]A. Spira, [2]S.A. Dunlop and [2]L.D. Beazley, [1]Faculty of Medicine, University of Calgary, Canada, [2]Department of Psychology, The University of Western Australia, Nedlands, 6009.

We have examined the projections of retinal ganglion cells (RGCs) in mature animals after the early removal of retino-recipient target tissue. By ablating target tissue at stages prior to RGC axon in-growth, we avoided damaging RGCs by axotomy. Mothers were sedated by inhalation of Halothane (5%) in oxygen/nitrous oxide. Pouch young between postnatal (P) days 8-10 were anaesthetised with Halothane, Xylocaine applied topically and a small slit made in the skin above the midbrain. Between one and three quarters of the right superibr colliculus was ablated thermally and the skin closed with isobutyl 2-cyanoacrylate (Ethicon). Animals recovered rapidly and were reared until between P120 and 130 when, in normals, the eyes are open, naturally occuring retinal ganglion cell death is complete and retinal projections are mature. The extent of the retinal projection was assessed after anterograde tracing from the left eye with horseradish peroxidase and wheat germ agglutinin (1:1, saturated solution in 0.5% triton under Halothane anaesthesia). After 2 days transport, animals were overdosed with Saffan (alphaxzolone, alphadolone acetate, Galxovet, 1ml/kg bw, im) and coronal vibratome brain sections (100 μm) processed with tetramethylbenzidine. Projections in experimental animals (n=10) were compared to normal (n=3). For animals with ablations that resulted in a loss of less than half the tectal volume, there was a normal pattern with crossed and uncrossed projections to the suprachiasmatic and accessory optic nuclei, lateral geniculate nucleus, pretectal complex and superior colliculus. In animals with deficits of between one half and three quarters of the tectal volume, projections to the suprachiasmatic, accessory optic and lateral geniculate nuclei were normal. However, projections to the pretectal area appeared expanded. In addition, the projection to the colliculus penetrated the deep layers and some axons crossed the midline to invade the ipsilateral colliculus. Projections to the inferior colliculus were also seen bilaterally. The loss of large amounts of target tissue appears to induce abnormal sprouting of RGC axons into both visual and non-visual nuclei.

Funded by the National Health & Medical Research Council.

Poster 16

RETINOTOPY IN THE DEVELOPING OPTIC NERVE OF THE WALLABY (*MACROPUS EUGENII*).

Y. Ding and L.R. Marotte
Developmental Neurobiology Group, R.S.B.S., A.N.U., Canberra, ACT 2601

Retinotopic order in the optic nerve was investigated in 38 wallaby pouch young aged 27-95 days. Animals were anaesthetised by either hypothermia or by intramuscular ketamine and xylazine (0.05-2 mg and 0.1-0.2 mg per 100g body weight, respectively) and a small piece of gelfoam impregnated with a carbocyanine dye was inserted into the peripheral retina. After 2-3 days survival animals were deeply anaesthetised as above, perfused with fixative and the optic nerve was sectioned transversely on a vibratome. At all stages of development axons showed a similar coarse retinotopy in the nerve. Axons from temporal retina entered the corresponding region of nerve at the optic nerve head as did axons from nasal, dorsal and ventral retina. Temporal axons shifted slightly dorsally away from the optic nerve head, occupying 43% of the cross sectional area of the nerve initially, decreasing to 25% from 41-47 days onwards. The region occupied by nasal axons shifted slightly more ventrally as distance from the optic nerve head increased and covered 49% of the cross sectional area of the nerve with little change at later ages. Dorsal axons gradually shifted ventrally through the central regions of the nerve to occupy a ventral position closer to the chiasm. Axons from ventral retina occupied a crescent shaped region ventrally close to the optic nerve head. Generally splitting into two groups, they shifted dorsally around the periphery of the nerve with increasing distance from the optic nerve head, to occupy a crescent shaped region dorsally, close to the chiasm. The density of axons within the labelled region decreased with age. Dorsal and ventral axons showed similar changes in occupation of cross sectional area to those of temporal axons. The timing of these relatively small changes did not correlate with the timing of the dramatic changes previously reported in the development of retinotopy of these axons in the superior colliculus (1).

1. Ding, Y. & Marotte, L.R. (1993) Proc.Aust.Neurosci.Soc. 4, 44.

ORA-5-5

RETINOTOPIC ORDER OF OPTIC AXONS DURING THE FORMATION OF THE RETINOCOLLICULAR PROJECTION IN THE WALLABY (*MACROPUS EUGENII*)

Y. Ding and L. Marotte
Developmental Neurobiology, Research School of Biological Sciences, Australian National University, Canberra ACT 2601

The degree of retinotopy present in the developing optic projection to the superior colliculus (SC) has been investigated in the wallaby by labelling small groups of axons from different retinal quadrants in animals from 12-95 days of age. Animals were anaesthetised by either hypothermia or by intramuscular ketamine and xylazine (0.05-2 mg and 0.1-0.2 mg per 100 g body weight, respectively) and a small piece of gelfoam impregnated with a carbocyanine dye was inserted into the retina. After 2-3 days survival animals were deeply anaesthetised as above, perfused with fixative and whole mounts of the retina and SC were made. At 12 days retinal axons are yet to reach the caudal pole and medial border of the SC, not doing so until 18 and 26 days respectively. Nevertheless axons were topographically organised even at this early stage with ventral axons distributed medially, dorsal axons laterally and temporal axons restricted to rostral SC. Between 42-52 days axons began forming terminal zones in their retinotopically correct regions. These were somewhat larger than their mature terminal zones. Prior to formation of the terminal zones axons showed little or no branching and over this time the branching was primarily close to or within the region of the terminal zone. By 65 days many of the initially more widely distributed axons had disappeared and by 95 days a discrete terminal zone was present. The developing projection in the wallaby appears to show a greater degree of topographic order than in the rat where axons from different retinal quadrants are distributed over much of the SC and branch widely early in development (1). The results in the wallaby show there is a protracted period during which axons continue arriving in the SC and are distributed in coarse topographic order before they begin forming terminal branches in their topographically appropriate position. Such a two stage process strongly suggests that shifting connections, a feature of the developing retinotectal projection of non-mammalian vertebrates, does not occur in this mammalian system.

1. Simon, D.K. & O'Leary D.D.M. (1992) J.Neurosci. 12, 1212-1232.

ORA-5-6

ONSET OF ELECTROPHYSIOLOGICAL RESPONSES TO STIMULATION OF THE OPTIC NERVE IN THE DEVELOPING SUPERIOR COLLICULUS OF THE WALLABY (*MACROPUS EUGENII*) : FURTHER EVIDENCE OF A TWO STAGE PROCESS OF FORMATION OF TOPOGRAPHICALLY ORDERED CONNECTIONS.

T. Freeman and R.F. Mark, Developmental Neurobiology Group, Research School of Biological Sciences, Australian National University, Canberra ACT 2601.

The electrophysiological development of the retinocollicular projection in the wallaby was studied with current source density analysis (CSD). Pouch young aged between 38 and 146 days were anaesthetised with ethyl carbamate (0.9ml/100g, 20% solution w/v intraperitoneally). The wave form of the response of the mature colliculus to stimulation of the optic nerve was negative on the surface and reversed with depth. CSD showed a short latency sink of synaptic activity deep in the colliculus followed by 3 closely timed sinks at more superficial levels. A response was first recorded at 40 days of age and consisted of a simple low amplitude wave with one reversal region, localised to the rostrolateral margin of the colliculus. In animals younger than 40 days there were no responses at all to stimulation of the optic nerve. Comparing these findings with those from the anatomical tracing of optic axons to the developing colliculus (1) we find that the onset of synaptic activity corresponds with the formation of terminal arborizations of optic axons. In development this follows a period of more than a month during which unbranched optic axons have grown into the colliculus and accumulated in large numbers and in correct retinotopic order. Therefore innervation of the colliculus by the optic nerve consists of two temporally separate processes; firstly a massive ingrowth of axons which are disposed in retinotopic order but with no evidence, either anatomical or physiological, of synapse formation or nerve conduction and secondly a stage of terminal branching of the axons coupled with the emergence of conduction in the optic nerve and evidence of synaptic transmission at terminals in the colliculus.

1. Ding, Y. & Marotte, L.R. (1993) These proceedings.

Poster No. 80

THE INITIAL DISTRIBUTION OF RETINAL AXONS ON THE SUPERIOR COLLICULUS AND THEIR SEQUENCE OF OUTGROWTH FROM THE RETINA IN THE WALLABY (*MACROPUS EUGENII*)

Y. Ding and L.R. Marotte
Developmental Neurobiology, Research School of Biological Sciences, Australian National University, Canberra ACT 2601

The distribution of ingrowing retinal axons over the superior colliculus (SC) and the order of their outgrowth from the retina has been investigated in the wallaby. The projection to the SC was labelled by an intravitreal injection of horseradish peroxidase (HRP). Retinal ganglion cells were retrogradely labelled in fixed tissue by a carbocyanine dye put into one lobe of the SC. Animals were deeply anaesthetised by hypothermia prior to eye injection or perfusion. Axons first reached the rostrolateral edge of the contralateral SC 4-5 days after birth. Ganglion cells extending these axons were extremely sparse and in the central region of dorsal retina. In the next few days axons expanded over the surface to cover approximately the most rostral three quarters of the SC but only in the lateral half and they were most dense rostrally. This extension of innervation caudally and the increased density rostrally correlated with the increased numbers of retrogradely labelled ganglion cells in nasal and particularly in temporal retina. There were very few labelled cells in ventral retina correlating with the lack of innervation of medial SC. As the innervation reached the medial edge of the SC by 26 days the number of labelled cells in ventral retina increased. Axons reached the rostrolateral edge of the ipsilateral SC a day or so later than the contralateral SC was first innervated and arose from the same dorsal and central region of retina that gave rise to the initial contralateral projection. They then spread in a caudomedial direction to cover the rostrolateral half by 14-16 days and by 46 days sparsely and transiently covered the SC. As early as 14-16 days ganglion cells supplying this projection were concentrated primarily in peripheral temporo-ventral retina, the region which projects ipsilaterally in the adult. The initial projection to the contralateral SC comes from appropriate regions of retina and is distributed in correct retinotopic order on the surface of the SC. From a very early age the projection to the ipsilateral SC arises predominantly from the retinal region appropriate for the adult projection.

191.11

SPECIFICITY OF THE RETINOCOLLICULAR PATHWAY IN DEVELOPING CAT AND FERRET. Cara J. Snider & Leo M. Chalupa*, Dept. of Psychology & Center for Neuroscience, University of California, Davis, CA 95616.

In carnivores as in other mammalian species the early retinal projection to the superior colliculus (SC) is considerably more widespread than at maturity (Williams & Chalupa, 1982). We have examined the topographic organization of this projection in developing cats and ferrets using focal implants or injections of the lipophilic tracer DiI in animals of known gestational ages. DiI deposits were made in preparations fixed with 4% paraformaldehyde as well as *in vivo*. Anterograde labelling patterns were visualized using fluorescent and confocal microscopy in wholemounts of the midbrain as well as in sagittal sections. Following small deposits of DiI into peripheral temporal retina the first contingent of fibers was observed entering the SC at gestation (G) age 37. This is at about the time that the peak number of fibers is attained in the developing optic nerve (Williams et at. 1986). By G42 a well-defined terminal zone of anterograde labelling was observed in both the contralateral and ipsilateral SC in the topographically appropriate region. In all cases, only a few fibers were seen to extend caudally and medially from the terminal zone; many of these ending in well defined growth cones. About one week later (G49), the terminal zone appeared adult-like with aberrant fibers rarely present. These observations are in marked contrast to the major topographic targeting errors observed with similar methods in the developing rat's contralateral retinocollicular projection by O'Leary and colleagues. Our results, in contrast, indicate a remarkable degree of precision in the developing carnivore retinocollicular pathway. (Supported by EY03391 from the NEI)

191.12

INCREASED SEROTONINERGIC INNERVATION IS ASSOCIATED WITH ABNORMAL RETINOTECTAL PROJECTIONS IN THE HAMSTER. R.W. Rhoades*, C.A. Bennett-Clarke and R.D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Anterograde tracing with HRP was used to compare the organization of retinotectal projections in normal adult hamsters and in animals that sustained subcutaneous injections of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) on the day of birth. Neonatal injection of this neurotoxin decreases the density of the serotoninergic (5-HT) innervation of the cerebral and cerebellar cortices, but increases the density of these fibers in the brainstem including the superior colliculus (SC). Analysis of tissue from the retinorecipient laminae of the SC by high-pressure liquid chromatography indicated that these lesions increased the amount of 5-HT in the adult by 47%. The increased serotoninergic innervation of SC was associated with a marked change in the distribution of the uncrossed retinotectal projection. In normal adult hamsters, fibers from the ipsilateral eye form dense clusters in the lowermost *stratum griseum superficiale* (SGS) and *stratum opticum* (SO). A small number of uncrossed fibers are also visible in the more caudal portions of these layers. In the animals that sustained neonatal 5,7-DHT injections, uncrossed retinotectal fibers formed a nearly continuous band in rostral SO and lower SGS and numerous labelled fibers were present in the caudal SC, primarily in the SO. Neonatal treatment with 5,7-DHT also produced alterations in the crossed retinotectal pathway. These results indicate that the 5-HT input to the developing brainstem may strongly influence the development of retinofugal projections.

Supported by EY 04170, EY 08861, and EY 08015

191.13

A TWO STAGE PROCESS IN THE DEVELOPMENT OF A MAMMALIAN RETINOCOLLICULAR PROJECTION: ANATOMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE. Y. Ding, T. Freeman, R.F. Mark* and L.R. Marotte. Developmental Neurobiology, RSBS, The Australian National University, Canberra, Australia.

The retinotopic order and onset of synaptic transmission in the developing optic projection to the superior colliculus (SC) has been investigated in the marsupial mammal, the wallaby. Small groups of axons from different retinal quadrants were labelled with DiI *in vivo* in animals from 12-95 days and responses to optic nerve stimulation recorded in the SC in animals from 38-146 days. At birth retinal axons have not reached the SC. At 12 days they are yet to reach the far caudal and medial borders of the SC. Nevertheless, axons are coarsely topographically organized even at this early stage. Between 42-52 days axons begin branching to form terminal zones in their retinotopically correct regions. Concomitant with this, the first evoked potentials to optic nerve stimulation can be recorded in the SC. By 65 days many of the initially more widely distributed axons have disappeared and by 95 days discrete terminal zones are present.

There is a protracted period during which axons continue arriving in the SC and are distributed in coarse topographic order with no evidence of synaptic transmission between retinal and collicular cells. They then begin forming terminal branches in their topographically appropriate position and this is coupled with the emergence of conduction in the optic nerve and synaptic transmission between retinal and collicular cells. Such a two stage process strongly suggests that shifting connections, a feature of the developing retinotectal projection of nonmammalian vertebrates, does not occur in the mammalian system.

191.14

ONTOGENY OF AMINO ACIDS IN SUPERIOR COLLICULUS AND VISUAL CORTEX. G.T. Golden*, G.G. Smith, F.A. Grau, P.F. Reyes, T.N. Ferraro. Department of Veterans Affairs Medical Center, Coatesville, PA 19320 and Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107

Recent studies suggest a role for amino acids (AAs) in the development of the nervous system. In the present study, an ultrasensitive gradient (two buffer) ion-exchange HPLC with post column derivitization was used to measure levels of 25 AAs and related amino compounds in Long-Evans rat superior colliculus (SC) and visual cortex (VC) at 15 developmental time points from post conception day (PCD) 15 through 33. Seventy-two time-mated dams were used to provide fetal and neonatal SC and VC. Newborn and fetal rat pups (removed from anesthetized dams) were weighed, C-R length recorded, sexed, euthanized and tissues dissected and frozen at -70° C in less than four minutes. Results showed highly significant positive correlations between body weight and C-R length, age and C-R length, age and body weight. There was a low negative correlation between body weight and litter size. Developmental AA profiles in SC and VC showed a close correspondence; however, the two regions could be distinguished on the basis of relative levels of GABA, glutamate and glycine (early fetal and late neonatal), taurine (late neonatal), lysine and histidine. Comparison of SC and VC levels of AAs before and after the day of birth (PCD 22) revealed that levels of many AAs including histidine, phosphoserine, phosphoethanolamine, phenylalanine, methionine, leucine, valine, tyrosine, glutamate, glycine, aspartate, and arginine decreased at birth but recovered to pre birth levels by PCD 23. Exceptions were phenylalanine, methionine, leucine and valine which maintained the lower levels postnatally up to PCD 33. B-alanine (SC only), GABA, glycine, glutamate and phosphoserine were at adult levels by PCD 33. Developmental AA profiles are discussed in terms of significant developmental events that occur in the rat visual system. Supported by the Department of Veterans Affairs.

191.15

DEVELOPMENT OF SUPERIOR COLLICULUS VISUAL RESPONSES AND THEIR CONTROL BY SUPRASYLVIAN CORTEX. B.E. Stein* and M.T. Wallace, Department of Physiology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA, 23298

Visually responsive neurons in the deep layers of the superior colliculus (SC) play important roles in attentive and orientation responses. The selectivity with which these neurons respond to visual stimuli and, thus, their likelihood of initiating overt responses, is controlled largely by inputs from lateral suprasylvian (LS) cortex (Ogasawara et al. 1984; Hardy and Stein 1988). In the present study we sought to determine: (a) when deep SC neurons first develop visual responses, and (b) when these neurons are first influenced by LS cortex. We examined the incidence and visual response properties of SC neurons in the cat at different ages (21 DPN-adult), and then studied the effects on these properties of reversibly deactivating (i.e., cooling) LS cortex. Deep layer SC neurons exhibit a surprisingly late maturation. Whereas a number of superficial layer neurons respond to visual stimuli in 6-7 DPN animals, no deep layer visual responses were seen until 21 DPN. A significant number (22% of the population) of visually-responsive neurons were not apparent in the deep layers until 35 DPN. The earliest visual responses in these neurons were not affected by LS deactivation. It was not until 28 DPN that some SC neurons showed influences from LS, and by 35 DPN the majority (60%) of visual neurons were affected by LS deactivation. These data are consistent with, and may help explain, the protracted developmental time course of visual attentive and orientation behaviors (Norton 1981; Sireteanu and Maurer 1982). They also underscore the critical developmental interplay between cortex and the SC. Supported by NIH grants NS08902 and EY06562.

191.16

VISUAL CORTICOTECTAL PROJECTION IN THE RABBIT: POSTNATAL DEVELOPMENT AND ITS MODIFICATION AFTER MONOCULAR DEPRIVATION. L.Martinez-Millán*, F.J. Sampedro, G. García del Caño and L.Gerrikagoitia. Dept. Neurosciences, Fac. of Medicine, University of the Basque Country, 48940-Leioa, Bizkaia, Spain.

Anterograde transport of iontophoretically administered biocytin in the Primary Visual Cortex of the rabbit was used to visualize the corticotectal connections in normal animals of PO, P3, P6, P7, P8, P11, P15, P20, P45 and adult stages. The same tracer was applied to 20 additional animals of P45, whose retinae were eliminated under deep anesthesia at postnatal day 2.

During the first postnatal week, the corticotectal connection consisted of thin and poorly ramified fibers, which distributed diffusely in the stratum opticum (SO), stratum griseum superficially (SGS) and stratum zonale (SZ). Most of these fibers were tangentially oriented with respect to the collicular surface and were more abundant in the lower half of the SGS. Superficial corticotectal fibers presented en passant swelling scattered along their length. At the beginning of the second postnatal week, corticotectal fibers showed an increased concentration in the posterolateral portion of the strata superficiale. At this stage a remarkable number of growth cones was detected in the superficial half of SGS. In subsequent days a higher number of fibers in the superficial half of SGS and SZ, together with a general increase of the en passant swellings, was observed. Towards the beginning of the third postnatal week, the corticotectal connection was organized into a column, containing vertical and oblique fibers, which ramified abundantly in the SZ.

In visually deprived animals, the corticollicular projection was very extensive in comparison with normal animals of the same age. The high density of fibers and en passant swellings always lay in the posterolateral sector of the SGS. At this level abundant oblique fibers and a high density of terminals in the superior half of the projection were observed. Furthermore, in these deprived animals, abundant fibers ran in the SZ and superior half of the SAGS from lateral to anteromedial direction reaching nearly the total extension of the superior colliculus (SC). At variable intervals along the length of all these fibers abundant en passant swellings were present.

neuroReport

NeuroReport 5, 117–120 (1993)

Two stages in the development of a mammalian retinocollicular projection

Richard F. Mark,[CA] Tobe C. B. Freeman,* Yuchuan Ding and Lauren R. Marotte

Developmental Neurobiology Group and Centre for Visual Sciences, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra ACT 2601, Australia

*Present address: University Laboratory of Physiology, Oxford OX1 3PT, UK

[CA] Corresponding Author

THE retinocollicular projection in the marsupial mammal the wallaby *Macropus eugenii*, has been investigated anatomically to determine the order in the developing projection and electrophysiologically to determine the time of onset of synaptic transmission by recording evoked potentials in the colliculus in response to stimulation of the optic nerve. There are two clear stages: a protracted period when retinal axons grow into the colliculus in coarse retinotopic order with no recordable electrical activity followed by the formation of terminal zones in retinotopically correct positions, the loss of more widely distributed axons and the onset of evoked potentials. The two stages are not seen in non-mammalian vertebrates where the projection is functional from the beginning.

Key words: Superior colliculus; Development; Retinotopy; DiI; Electrophysiology; Mammal; Marsupial

Introduction

A direct neuronal connection from the eye to the contralateral midbrain is a feature of all vertebrates. Its development has been exhaustively studied, both anatomically and electrophysiologically, in the fish and amphibia as a model of the formation of topographically ordered maps.[1] From the time retinal axons first reach the tectum in these animals the projection shows some degree of retinotopic organization[2–5] and is functional.[6] Much less is known of the development of the homologous retinocollicular projection in mammals. An anatomical study in the rat[7] suggests that the projection is more diffusely ordered initially and precision is achieved by the elimination of many misplaced branches and arborizations. At what stage functional connections are formed during the process in mammals is not known. We report here, for the first time in a mammal, a combined electrophysiological and anatomical analysis of the developing retinocollicular projection in a marsupial, the wallaby *Macropus eugenii*. The aim was to investigate the retinotopic order in the projection during development and the stage at which terminal zones formed during this process using anatomical methods and to correlate this with the onset of functional connections between retinal and tectal cells by recording evoked potentials in the superior colliculus (SC) to stimulation of the optic nerve.

Our results show two stages in the development of the projection: a period when retinal axons grow into the SC and are distributed in rough retinotopic order with little or no branching and no evidence of functional connections between retinal and tectal cells followed much later by the formation of terminal axonal arborizations in their retinotopically correct positions coupled with evidence of functional connections.

Materials and Methods

Anatomy: The retinotopy of the developing projection in the SC was investigated in 43 wallabies (*Macropus eugenii*), aged 27–95 postnatal days, by labelling small groups of axons from different retinal quadrants with the carbocyanine dye 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), Molecular Probes. Animals younger than 35 days were anaesthetized by hypothermia while older animals were anaesthetized by intramuscular injection of 1–3 mg 100 g^{-1} body weight of ketamine and 0.2 mg 100 g^{-1} body weight of xylazine. A small piece of Gelfoam (Upjohn), impregnated with a concentrated solution of DiI dissolved in ethanol and then dried, was inserted into peripheral retina. After 1–5 days survival, depending on age, wholemounts of retina and SC were prepared and examined[7] and then mapped with Neurotrace software (InterActions Company, P.O. Box 953, Kendall Square, Cambridge, MA 02142, USA).

Electrophysiology: Twenty-four pouch young aged between 38 and 146 days were used. They were anaesthetized with 20% ethyl carbamate (0.9 ml 100 g^{-1} body weight) intraperitoneally. One-quarter of the initial dose was administered hourly or more frequently as required to maintain anaesthesia. The electrocardiogram was monitored continuously. Animals were placed in an artificial pouch at 37°C with the head fixed. Bipolar stimulating needle electrodes with tip

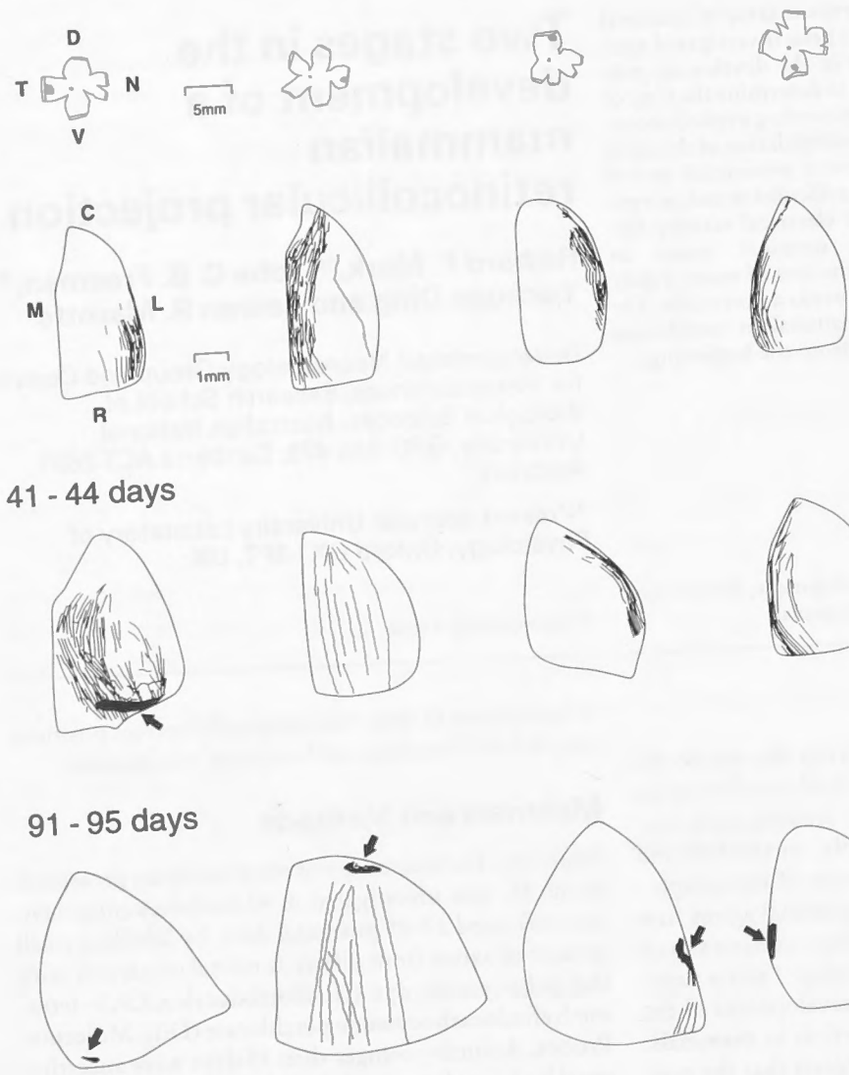


FIG. 1. The distribution of labelled retinal axons and terminal zones in contralateral SC after deposits of DiI in temporal, nasal, dorsal and ventral quadrants of the retina is shown at various ages. Above are camera lucida drawings of retinal wholemounts at 27–29 days after birth. DiI deposits are in solid black. Spots mark the optic nerve head. T: temporal; N: nasal; D: dorsal and V: ventral. Deposits were in similar positions for other ages. Below are camera lucida drawings of wholemounts of the SC. R: rostral; C: caudal; M: medial; L: lateral. Sizes vary slightly at similar ages because of flattening to produce the wholemounts. Not every labelled axon is drawn but the rostrocaudal and mediolateral extent of the labelled area and any solitary axons outside it are shown. Terminal zones are solid black and arrowed. Prior to 41 days axons had few branches and showed a coarse retinotopy, with axons from temporal retina rostrally, nasal axons reaching to the caudal pole, dorsal axons laterally and ventral axons positioned medially. At 41–44 days terminal branching was first seen after labelling temporal but neither nasal, dorsal nor ventral axons. This was in retinotopically correct rostral SC. By 55 days axons from other retinal quadrants had begun to form terminal zones in their retinotopically correct positions. By 91–95 days discrete terminal zones were present.

separation of 200 μm were placed by direct vision on the optic nerve head after removal of the lens and vitreous humor. Stimuli were 1 ms square waves, 0–30 volts, repeated every 2 or 3 s. A glass microelectrode filled with 2 M sodium acetate (0.5 MΩ) was inserted through a drill hole in the skull onto the SC. Recordings were made from the surface of the SC and at increasing depths in steps of 40 μm until the response reversed from the initial negative wave to a positive wave of maximum amplitude at deep recording positions. Electrode advance was by a programmable piezo-electric drive which eliminated tissue drag (Burleigh Instruments). Recordings were collected, averaged and stored in a PC with software designed by Small Systems Software Innovations, Australia. After recording a lesion was made (5 microamps for 10 s) and its position in the SC confirmed histologically.

Results

The mature retinocollicular projection in the wallaby follows the retinotopic organization common to non-primate mammals with laterally placed eyes.[8] Retinal axons first reach the rostrolateral edge of the SC 4 days and the caudal pole 26 days after birth.[9] Labelling axons from different retinal quadrants with a fluorescent dye showed that at 27–29 days, retinal axons were already distributed in a coarse retinotopic order (Fig. 1). They ran primarily in a rostrocaudal direction and many were tipped with growth cones. There was little branching and that seen was in the form of short side branches. Axons labelled from temporal peripheral retina were distributed rostrally and did not extend into more caudal SC. Nasal axons extended across the SC to the caudal pole. Depending on whether the position of the dye deposit was slightly dorsal or ventral in the nasal retina, labelled axons could course medially as in the example shown, across the entire mediolateral extent or laterally to reach caudal SC. Dorsal axons extended from rostral to caudal along the lateral SC and ventral axons extended in a similar manner along the medial border. Axons

continued to accumulate in the SC but this was the state of the innervation until 40 days.

Beginning at 41–44 days temporal axons began branching to form a terminal zone in their retinotopically correct area in rostral SC (Fig. 1) and by 55 days axons from other retinal quadrants had also begun to form terminal zones in their retinotopically appropriate regions. These were somewhat larger than their mature terminal zones (Fig. 1) and the more widely distributed labelled axons were still prominent. At 91–95 days discrete terminal zones were present and the more widely distributed axons had disappeared (Fig. 1), confirming the time of attainment of precision determined by other methods.[10,11]

Concomitant physiological experiments showed that at 38 days there was no electrical activity recorded from the SC in response to stimulation of the optic nerve head (Fig. 2). The first response was recorded at 42 days in the form of a negative wave just discernible above the baseline noise rostrally and a similar sized positive wave caudally. Neither potential reversed with depth. By 45 days the negative potential on the surface at the rostral pole reversed to positive deeper in the SC, as does the evoked potential in older animals. By 55 days a larger evoked potential could be recorded from the rostral half of the colliculus and it reversed polarity with depth. This was still accompanied by the positive wave further caudally in the colliculus, which did not reverse with depth. Between this age and 87 days the area of SC from which a reversing potential could be recorded spread caudally and by 87 days it was found over the whole SC. It showed several waves, characteristic of the mature projection[12] (Fig. 2).

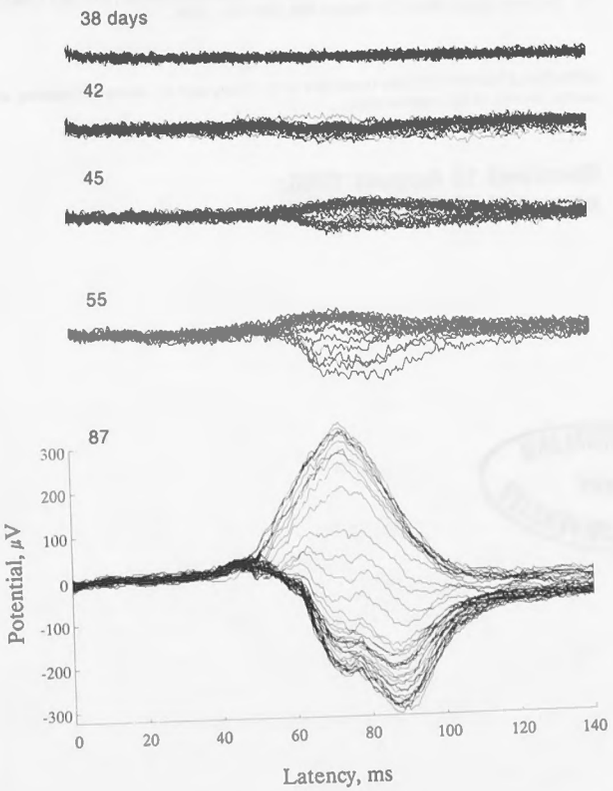


FIG. 2. The potentials evoked in the rostral SC of pouch young wallabies following stimulation of the optic nerve head are shown at increasing ages. Each single trace is the average of 10 sweeps. Between each trace the electrode was advanced 40 μm deeper into the SC. Low frequency cut-off was 0.5 Hz. There was no response at 38 days and a barely discernible movement from the baseline at 42 days. By 45 days a surface negative wave was recorded rostrally that reversed to positive more deeply, indicating the appearance of synaptic potentials of local origin. At 55 days the reversing wave was clear. By 87 days the waves began to show inflections characteristic of the mature evoked potential (the latter not illustrated).

Discussion

The onset of evoked potentials in the SC after stimulation of the optic nerve clearly delimits two stages of innervation of optic axons. There is a protracted period when axons grow into the SC in increasing numbers, show little branching and are distributed in coarse retinotopic order, all in the absence of recordable electrophysiological activity. The beginning of the second stage is signalled anatomically by the first elaboration of terminal branches of temporal optic axons at the rostrolateral colliculus and physiologically by the recording of evoked potentials to optic nerve stimulation in this region.

The first potentials were scarcely above background noise even with much averaging and were negative rostrally and positive further caudally and did not reverse with depth in either location, which is compatible with their being caused by the beginning of conduction of impulses along the unmyelinated optic axons in the stratum opticum of the SC.[13] A few days later the rostral potentials were found to reverse in polarity with depth in the SC. This is due to the generation of synaptic potentials in the collicular neurones orientated with the long axis orthogonal to the surface of the SC.[14] Responses must have been mediated by direct retinocollicular axons as the indirect pathway via the cortex does not appear anatomically until after 85 days and is not functional until after 135 days.[15] The onset of functional connections occurs well before the time when light-induced responses can be produced, as photoreceptor outer segments do not begin to form in central retina until 85 days after birth and in peripheral retina until 105 days and the eyes do not open until 140 days after birth.[16]

The first stage in the formation of this retinocollicular projection is not seen in non-mammalian vertebrates. The latter do not demonstrate a long period when optic axons invade the tectum in the absence of functional connections between retinal and tectal cells. Instead, functional synapses are made immediately[2,6] and it is because of this that a mechanism of shifting connections between retinal and tectal cells during development[1,3,17,18] is required to maintain retinotopic order in the face of incongruent modes of growth between retina and tectum.[1,18] The mammalian strategy demonstrated here, by which axons are first distributed

in a coarse order and then make functional connections in their correct positions, obviates this requirement. Besides, incongruent modes of growth of the retina[19,20] and colliculus[21,22] are not marked features of mammalian development.

Retinocollicular axons in the rat appear to go through a stage during the development of the projection which is not seen in the wallaby. Although they do tend to branch and arborise preferentially at their retinotopically correct position they also do so at retinotopically incorrect locations.[7] Misplaced branches and terminal arbors are then removed. The morphological stages in the process in the wallaby appear more similar to those described for retinal axons in the developing SC of the mouse[23] and hamster[24] and in the lateral geniculate nucleus of the cat.[25] Axons take relatively straight paths and make short side branches, followed later in development by the elaboration of usually a single terminal arborization. Whether arborizations are made in retinotopically correct positions could not be directly determined in these species but in the wallaby we have shown that they are.

Conclusion

Anatomical and electrophysiological investigation of the developing retinocollicular projection in the wallaby shows two stages in its formation. In the first stage retinal axons grow into the SC, are distributed in rough retinotopic order with little branching, show no signs of terminal zone formation and no electrically recordable functional connections exist between retinal and collicular cells. In the second stage axons form terminal zones at their retinotopically correct sites, more widely distributed axons disappear and functional synaptic connections are made. This process is completed some weeks before eye opening at 140 days of age.

References

1. Udin SB and Fawcett JW. *Ann Rev Neurosci* **11**, 289–327 (1988).
2. Holt CE and Harris WA. *Nature* **301**, 150–152 (1983).
3. O'Rourke NA and Fraser SE. *Devel Biol* **114**, 265–276 (1986).
4. Sakaguchi DS and Murphey RK. *J Neurosci* **5**, 3228–3245 (1985).
5. Kaethner RJ and Stuermer CAO. *J Neurosci* **12**, 3257–3271 (1992).
6. Chung S-H, Keating MJ and Bliss TVP. *Proc R Soc Lond B* **187**, 449–459 (1974).
7. Simon DK and O'Leary DDM. *J Neurosci* **12**, 1212–1232 (1992).
8. Mark RF, James AC and Sheng X-M. *J Comp Neurol* **330**, 303–314 (1993).
9. Ding Y and Marotte LR. *Proc Aust Neuroscience Soc* **4**, 44 (1993).
10. Marotte LR. *J Comp Neurol* **293**, 524–539 (1990).
11. Marotte LR. *J Comp Neurol* **331**, 1–13 (1993).
12. Freeman T and Mark RF. *Proc Aust Neuroscience Soc* **4**, 44 (1993).
13. Lorente de No R. *A Study of Nerve Physiology.* The Rockefeller Institute for Medical Research, 1947; Volume 132, Part 2, Chapter 16.
14. Freeman B and Singer W. *J Neurophysiol* **49**, 1075–1091 (1983).
15. Sheng X-M, Marotte LR and Mark RF. *J Comp Neurol* **300**, 196–210 (1990).
16. Spira AW and Marotte LR. *Anat Embryol* **179**, 571–585 (1985).
17. Easter SS Jr and Stuermer CAO. *J Neurosci* **4**, 1052–1063 (1984).
18. Gaze RM, Keating MJ, Ostberg A *et al. J Embryol Exp Morph* **53**, 103–143 (1979).
19. Walsh C and Polley EH. *J Neurosci* **5**, 741–750 (1985).
20. Harman AM and Beazley LD. *Neurosci* **28**, 219–232 (1989).
21. Cooper ML and Rakic P. *J Comp Neurol* **202**, 309–334 (1981).
22. Harman AM. *J Comp Neurol* **313**, 469–478 (1991).
23. Sachs GM, Jacobson M and Caviness VS Jr. *J Comp Neurol* **246**, 395–408 (1986).
24. Jhaveri S, Edwards MA and Schneider GE. *Exp Brain Res* **87**, 371–382 (1991).
25. Sretavan D and Shatz CJ. *Nature* **308**, 845–848 (1984).

ACKNOWLEDGEMENTS: We thank Drs S.-H. Chung and W. Harris for reading an earlier version of the manuscript.

Received 16 August 1993; accepted 19 August 1993